MEDICINSKA FAKULTETA

DOKTORSKA DISERTACIJA

UPORABA DINAMIČNE MAGNETNORESONANČNE PREISKAVE V SLEDENJU VRAŠČANJA REKONSTRUIRANE KOLENSKE SPREDNJE KRIŽNE VEZI PRI INTRAOPERATIVNI APLIKACIJI RASTNIH FAKTORJEV

Mitja Rupreht

Februar 2012

UNIVERZA V MARIBORU

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Mitja Rupreht

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September 2011

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POVZETEK

Ozadje. Raztrganina sprednje križne vezi (Anterior cruciate ligament, ACL) spada med najpogostejše poškodbe kolena. Po rekonstrukciji je zaradi počasnega vraščanja in ligamentizacije presadka rehabilitacija dolgotrajna. Z namenom pospešiti vraščanje potekajo študije različnih modifikacij rekonstrukcije, v zadnjem desetletju najpogosteje z lokalno apliciranim trombocitnim gelom (TG, Platelet rich plasma gel, PRPG) kot nosilcem različnih rastnih faktorjev (RF). Zaradi etičnih in tehničnih razlogov je histološko ugotavljanje učinkov TG pri človeku praktično nemogoče, predvsem pri vraščanju v oba tunela, zato sledenje poteka neinvazivno z uporabo magnetne resonance (MR). **Namen.** Prvi namen raziskave je bil ugotoviti uporabnost novejših kvantitativnih MR metod, slikanja z difuzijsko obtežitvijo (Diffusion weighted imaging, DWI) in dinamičnega kontrastnega poudarjenega slikanja (Dynamic contrast enhanced imaging, DCEI), v kvantitativni oceni edema in prekrvljenosti v proksimalnem delu tibialnega tunela, ki je predstavljal interesno področje (Region of interest, ROI). Drugi namen raziskave je bil uporabiti obe kvantitativni MR metodi za oceno morebitnih učinkov intraoperativno lokalno apliciranega TG na edem in prekrvljenost v interesnem področju. **Metode.** Pri petdesetih bolnikih je bila opravljena artroskopska rekonstrukcija ACL. Testna skupina 25 bolnikov je intraoperativno lokalno prejela TG, medtem ko ga kontrolna skupina preostalih 25 bolnikov ni prejela. Pri vsakem bolniku smo opravili MR preiskavo en, dva in pol ter šest mesecev po posegu. Z DWI in DCEI smo pregledovali proksimalni del tibialnega tunela. Pri vsaki DWI preiskavi smo izračunali vrednost navideznega difuzijskega koeficienta (Apparent diffusion coefficient, ADC) kot merila za mobilnost vodnih molekul. Pri ocenjevanju prekrvljenosti smo pri vsaki DCEI preiskavi izračunali dva parametra: a) gradient obarvanja (G) kot merilo za hitrost obarvanja s paramagnetnim kontrastnim sredstvom (KS), in b) faktor obarvanja (F) kot merilo za jakost

obarvanja. **Rezultati.** Povprečne vrednosti ADC kot tudi G in F so v obdobju sledenja v obeh skupinah med posameznimi kontrolnimi preiskavami značilno upadale. Primerjava skupin kaže, da je bila povprečna vrednost ADC v skupini, ki je prejela TG, en mesec po posegu 1,41 ($10^{-3}mm^2/s$) in značilno nižja kot v kontrolni (1,50 ($10^{-3}mm^2/s$)) skupini (p=0,033). Pri drugi in tretji kontroli razliki v povprečnih vrednostih ADC med skupinama nista bili značilni. Povprečna vrednost G je bila v testni skupini po enem (2,07 %/s) ter dveh in pol (1,64 %/s) mesecih značilno višja kot v kontrolni skupini (1,41 %/s po enem ter 1,15 %/s po dveh in pol mesecih, p=0,019 in p=0,008), pri tretji kontroli pa razlika ni bila značilna. Razlike v izračunanih povprečnih vrednostih F med skupinama pri nobeni kontrolni preiskavi niso bile značilne. **Zaključek.** Značilno nižja vrednost ADC v proksimalnem tibialnem tunelu skupine, ki je prejela TG, en mesec po rekonstrukciji, kaže na zmanjšanje mobilnosti vodnih molekul v tem področju v primerjavi s kontrolno skupino, kar pripisujemo gostejši celični organizaciji. Značilno višja vrednost G v skupini, ki je prejela TG, en ter dva in pol meseca po posegu, kaže na večjo gostoto krvnih žil v pregledanem področju v primerjavi s kontrolno skupino. Oboje dokazuje zgodnja učinka delovanja rastnih RF in drugih bioaktivnih proteinov v TG kot povečanje števila celic in večjo prekrvljenost v tibialnem tunelu, kar je v skladu s histološkimi opisi učinka RF na živalskih modelih. Kolikor vemo, gre za prvo uporabo DWI ter DCEI MR preiskave v področju ACL. Glede na rezultate naše raziskave ju zato predlagamo kot dodatni kvantitativni metodi ocenjevanja prihodnjih, hitro se razvijajočih modifikacij rekonstrukcije ACL.

Quantitative Evaluation of the Tibial Tunnel after Intraoperatively Locally Administered Platelet Rich Plasma Gel during Anterior Cruciate Ligament Reconstruction Using Diffusion Weighted and Dynamic Contrast-Enhanced MRI

ABSTRACT

Background. Anterior cruciate ligament (ACL) rupture is one of the most common knee injuries. After reconstruction, slow processes of graft healing and ligamentisation require long rehabilitation period. Many modifications of the reconstruction technique have evolved over the time, among them in last decade the local application of platelet rich plasma gel (PRPG) as the carrier of various growth factors (GF). For ethical and technical reasons, histologic evaluation of ACL graft incorporation as well as of the effects of modifications of ACL reconstruction in humans is impossible. Therefore, their assessment has been performed mostly with magnetic resonance imaging (MRI). **Purpose.** The first purpose of the study was to assess the feasibility of two new quantitative MRI methods, diffusion weighted imaging (DWI) and dynamic contrast-enhanced imaging (DCEI), in the quantitative assessment of the oedema and vascularity in the proximal tibial tunnel. The second purpose was to assess the effect of locally applied PRPG during ACL reconstruction using two quantitative MRI sequences, DWI and DCEI, for the quantitative measurement of the tibial tunnel oedema and vascularity. **Methods.** In fifty patients, ACL reconstructions were performed by standard arthroscopic procedure. They were divided in two groups. The patients in the PRPG group locally received an application of PRPG into the tibial and femoral bone tunnels and into the graft itself, whereas patients in the control group did not receive any PRPG. For each patient, MRI examinations were performed one, two and a half and six months after the procedure.

The proximal tibial tunnel was examined by DWI and DCEI, which were utilized to calculate apparent diffusion coefficient (ADC) values, which provide information on molecular motion of water, and the contrast enhancement gradient (G) and enhancement factor (F) values, which describe the enhancement rate and static enhancement after intravenously (i.v.) administered paramagnetic contrast medium (CM). **Results.** Calculated values of ADC, G and F in each group decreased significantly between the control examinations. In comparing the two groups, we find that at one month average ADC in the PRPG (1,41 ($10^{-3}mm^2/s$)) group was significantly lower than in the control (1,50 ($10^{-3}mm^2/s$)) group (p=0.033). At the second and third postoperative controls, the differences in the ADC values were not significant. At one month, average G was significantly higher in the PRPG (2,07 %/s) group than in the control (1,41 %/s) group (p=0.019). Also, at two and a half months, G was also significantly higher in the PRPG (1,64 %/s) group than in the control (1,15 %/s) group (p=0.008). At six months, there was no significant difference in the G between the groups. There were no significant differences in average F between the groups at any control examination. **Conclusion.** DWI and DCEI MRI proved to be feasible in noninvasive quantitative assessment of the oedema and vascularity in the healing process of ACL graft. In the proximal tibial tunnel, the effect of PRPG, measured with these two quantitative MRI methods, is mostly pronounced at early stages of the healing process. The significant decrease of ADC demonstrates diminished water mobility, presumably due do larger cellularity, in the first postoperative month. The significant increase of the G at one and two and a half months after reconstruction indicates higher vascular density as effect of the locally applied PRPG. Both results are in accordance with early histologic findings of enhanced fibrosis and angiogenesis as effects of intraoperatively administered GF in animal models. To our knowledge, this is the first time DWI and DCEI have been demonstrated in the area of ACL. We propose them as

additional quantitative MRI modalities in the assessment of new, rapidly evolving modifications of ACL reconstruction technique.

UDK: 616.72/.73-073-089.8:615.35(043.3)

KLJUČNE BESEDE: rekonstrukcija sprednjega križnega ligamenta, vraščanje, trombocitni gel, magnetna resonanca, difuzijsko obteženo MR slikanje, dinamično kontrastno poudarjeno MR slikanje

KEY WORDS: anterior cruciate ligament reconstruction, healing, platelet gel, MRI, diffusion-weighted MRI, dynamic contrast-enhanced MRI

1. UVOD

1.1. Raztrganina kolenske sprednje križne vezi, rekonstrukcija in vraščanje presadka

Sprednja križna vez (Anterior Cruciate Ligament – ACL) je glavni sprednji stabilizator kolena. Izhaja iz medialne fasete lateralnega femoralnega kondila, poteka v dveh snopih, anteromedialnem in posterolateralnem, v anteromedialni smeri in narašča tik pred interkondilarno eminenco tibialnega platoja.

Raztrganina ACL je ena najpogostejših poškodb kolenskega sklepa. Nastane kot posledica hiperekstenzijskga ali rotacijskega mehanizma. Kaže se predvsem kot sprednja nestabilnost, pogosto pa jo spremljajo poškodbe meniskusov ali sklepnega hrustanca, le te pa lahko nastanejo tudi kot posledica nezdravljene raztrganine, ki tako vodi v zgodnjo artrozo.

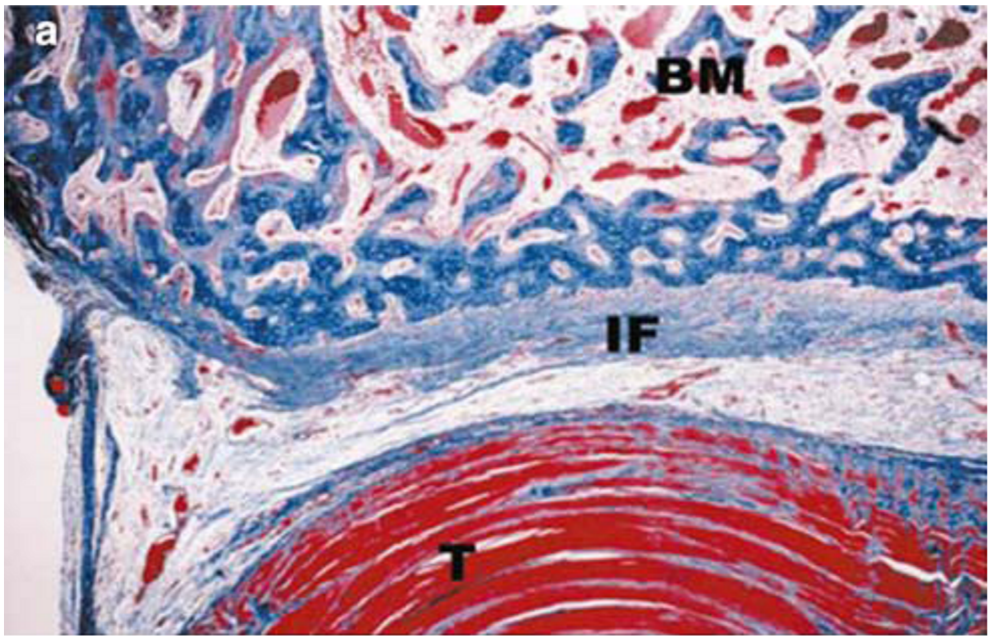
Kljub možnostim konzervativnega zdravljenja ter ekstraartikularnim posegom je danes intraartikularna rekonstrukcija uveljavljena terapevtska metoda predvsem pri mladi in aktivni populaciji (1). Le-ta poveča stabilnost kolena ter zmanjša možnost pozne raztrganine meniskusa in operativnih posegov (1-5). Kot transplantat je bil v preteklosti največkrat uporabljen kostnotetivni (bone-tendon-bone, BTB) patelarni ligament, v zadnjih letih pa vse bolj prevladuje uporaba tetiv mišic semitendinosus in gracilis ter fiksacija z resorbilnimi vijaki (6-8).

Po rekonstrukciji ACL potekata dva procesa: vraščanje presadka v femoralni in tibialni tunel ter zorenje oz. ligamentizacija intraartikularnega dela presadka (9).

Histološki opisi normalnega vraščanja presadka v tibialni tunel se nekoliko razlikujejo (8, 10, 11). Gre za kompleksen proces, odvisen od različnih kirurških in postoperativnih spremenljivk: tipa presadka (avto ali alograft, mehkotkivni in kostnotetivni presadek), načina

njegove fiksacije, napetosti, dolžine presadka v tunelu, razlike med premerom tunela in presadka in končno tudi od usmeritve tunela (9). V grobem proces poteka v več fazah: faza akutnega vnetja se začne takoj po popustitvi Esmarchove preveze, ko se koleno napolni s krvjo iz femoralnega in tibialnega tunela. Agregacija trombocitov povzroči sproščanje citokinov, kot so transformirajoči rastni faktor (RF) ß (transforming growth factor-ß, TGF-ß) in trombocitni rastni faktorji (platelet-derived growth factors, PDGF). Sledi kemotaksa nevtrofilcev in monocitov, njej pa provnetna citokinska kaskada. Nevtrofilcev je največ po 24-48 urah. Sledijo jim rekrutirani makrofagi, ki so potrebni za zorenje koagula in tvorbo zgodnjega granulacijskega tkiva, že 10 dni po rekonstrukciji pa so identificirali tudi lokalne makrofage (12). V fazi revaskularizacije monociti sprožijo angiogenezo, ki jo pospešujejo lokalna hipoksija in visoka koncentracija dušikovih oksidov, žilnega endotelijskega rastnega faktorja (vascular endothelial growth factor, VEGF) in fibroblastnega rastnega faktorja 2 (fibroblast growth factor-2, FGF-2). V fazi tvorbe ekstracelularnega matriksa fibroblasti razgrajujejo zgodnje granulacijsko tkivo oz. provizorični matriks z metaloproteinazami (MMP), hkrati pa tvorijo nov zunajcelični matriks oz. vezivnotkivno brazgotino, kar lahko traja več tednov. V fazi remodeliranja tumorski rastni faktor beta 1 (tumor growth factor-ß1, TGF- ß1) stimulira fibroblaste k tvorbi kolagena, predvsem tipov I,III,V, proteoglikanov in ostalih komponent ECM. Pri tem posredujejo citokini, ki uravnavajo delovanje serinskih proteaz in MMP. Pri zajcih se niti kolagena v vmesni coni med presadkom in steno kanala pojavijo po 4 tednih (Slika 1).

Slika 1. Vraščanje presadka v proksimalni del tibialnega tunela 4 tedne po rekonstrukciji.



Komentar k sliki 1: T tetiva, IF vmesna cona, BM kostni mozeg

Vir: Yamakado K, Kitaoka K, Yamada H et al (2002) The influence of mechanical stress on graft healing in a bone tunnel. Arthroscopy;18:82–90.

Celični in hormonski dejavniki, ki med fiziološko kaskado vraščanja presadka in tvorbe okolne kostnine igrajo pomembno vlogo, so še: trombocitni epidermalni RF, inzulinski RF, trombocitni factor angiogeneze, interlevkin-8, tumorski nekrotični faktor-α, vezivnotkivni RF, keratinocitni RF ter angiopoetin-2 (12, 13). Ti proteini imajo pozitivni efekt na proliferacijo fibroblastov, sintezo proteinov zunajceličnega matriksa, kot tudi na angiogenezo, in tako pospešujejo vraščanje (14-16). Največja ekspresija fizioloških RF je pri psih opisana 3 tedne po rekonstrukciji, njihova imunoreaktivnost pa upade na predoperativno raven po 12 tednih (17).

Vlakna, podobna Sharpeyevim, najdemo po 12 tednih. Po 6 tednih se število fibroblastov manjša, kolagenska vlakna pa se debelijo in penetrirajo v presadek in okolno kost ter tako tvorijo osteoligamentozno insercijo (Slika 2). (9, 18-23).

Slika 2. Histološki prerez tibialnega tunela v sagitalni ravnini pri psu 6 tednov po rekonstrukciji.

Komentar k sliki 2: Centralno je viden mehkotkivni presadek (T), med njim in kostjo pa vmesna cona, izpolnjena z granulacijskim tkivom.

Vir: Tomita F, Yasuda K, Mikami S et al. Comparisons of intraosseous graft healing between the doubled flexor tendon graft and the bone-patellar tendon-bone graft in anterior cruciate ligament reconstruction. Arthroscopy. 2001; 17:461–76.

Poleg tega se v tem času v tunelih začenja tudi neohondrifikacija in neoosifikacija (11). Nastajata hrustanec in kostnina, ki tvori sklerotični rob tunela. Sama osteoblastična aktivnost v tunelu lahko traja dlje, pri 29% bolnikov je osteoblastična aktivnost v tunelu zvišana tudi po 2 letih (24).

Proces intraartikularnega zorenja oz. ligamentizacije presadka se prav tako razlikuje glede na tip presadka. Pri BTB presadku se začne z nekrozo kostnega dela presadka,medtem ko intraartikularni del preživi in se zgodaj revaskularizira (25-27). Pri mehkotkivnih presadkih, torej tudi takšnih iz tetiv hamstringov (semimembranosus, semitendinosus in gracilis), pa znakov nekroze ni (18, 24, 28). V obeh primerih sledi remodeliranje intraartikularnega dela presadka, pri katerem so velike kolagenske fibrile nadomeščene z majhnimi (29,30). Presadek je najmočnejši neposredno po operaciji, med petim in šestnajstim tednom po operaciji pa je šibkejši zaradi nekroze in zgodnje revaskularizacije. Po tem obdobju postane bolj čvrst, vendar nikoli ne doseže čvrstosti tetive pred rekonstrukcijo (18, 31, 32).

Preizkus pretrganja pokaže, da je do 12. tedna le-to posledica izpuljenja tetive iz tunela oz. iz kosti, po 12. tednu pa posledica pretrganja intraartikularnega dela tetive (18,24). Izgleda, da tkivo dozori po 26 tednih, vendar to velja za živalske modele in tetivni presadek (13, 18, 24). Zaradi očitnih tehničnih omejitev je znanje o načinu in hitrosti vraščanja pri človeku omejeno. V literaturi lahko najdemo le prikaze primerov (33,34) in majhne serije (28,35), zato je pri direktni primerjavi z živalmi, tudi zaradi vprašljive primerljivosti hitrosti metabolizma s humanim, potrebna previdnost. Živalski modeli so sicer pokazali hitrejše vraščanje presadka

patelarnega ligamenta (bone-patellar tendon-bone, BTB) (18, 36), vendar pa je to povezano z zapleti na mestu odvzema, ki so težji v primerjavi z rekonstrukcijo s tetivami hamstringov (37,38).

1.2. Poskusi vplivanja na vraščanje presadka

Zaradi zelo počasnega vraščanja presadka v okolno spongiozno kost je rehabilitacija po posegu dolgotrajna, saj bolnikom niso dovoljene polne obremenitve prej kot 6-9 mesecev po njem. Zato raziskovalci preizkušajo različne metode rekonstrukcije, da bi pospešili vraščanje. Na živalskih modelih je bilo histološko dokazano hitrejše vraščanje pri lokalni aplikaciji mezenhimskih matičnih celic (39), kalcijevega trifosfata (40) ter pri hiperbarični oksigenaciji (41). Pri uporabi teh metod so med 4. in 8. tednom po rekonstrukciji našli več zrelega vezivnega in kalciniranega hrustanca (39-41) in izrazitejšo neovaskularizacijo (41) v vmesni coni ter zvečano silo izpuljenja (40).

Z namenom pospešiti vraščanje je bilo opravljeno obsežno raziskovanje učinka lokalne aplikacije različnih kombinacij RF, večinoma v obliki trombocitnega gela, na poskusnih živalih. Študije, ki to preučujejo, temeljijo na histoloških analizah ter meritvah sile pretrganja (42-50).

V zadnjem desetletju potekajo podobne raziskave na ljudeh, kjer pa invazivne metode sledenja niso možne, zato le-to poteka v glavnem z MR ter mehaničnimi testi (51-54).

1.3. Plazma, bogata s trombociti, njeni priprava in aktivacija ter nastanek trombocitnega gela

Trombociti so citoplazmatski fragmenti megakariocitov, formiranih v kostnem mozgu. V procesu zorenja megakariocitov se v njih tvori več tipov zrnc oz. granul. Med njimi so najpomembnejša α zrnca, ki vsebujejo več kot 30 bioaktivnih beljakovin, med katerimi imajo mnoge ključno vlogo v procesih hemostaze in celjenja. Med njimi so najpomembnejši PDGF, ki imajo, kot rečeno, vlogo pri aktivaciji makrofagov, angiogenezi, stimulaciji fibroblastov, sintezi kolagena in proliferaciji osteoblastov. Poleg njih so pomembni tudi TGFβ1 kot stimulator fibroblastov in biosinteze kolagena ter inhibitor osteoklastov, inzulinski RF kot stimulator kemotakse fibroblastov in diferenciacije osteoblastov, trombocitni endotelijski RF kot stimulator celjenja ran s pospeševanjem proliferacije keratocitov in dermalnih fibroblastov, endotelijski RF in trombocitni faktor angiogeneze s stimulacijo žilnih endotelnih celic. Trombocitni faktor 4 stimulira začetni prehod nevtrofilcev v področje rane z antiheparinskim učinkom, VEGF pa stimulira angiogenezo, tvorbo lumna malih žil in vazodilatacijo (28).

Plazma, bogata s trombociti (PBT, platelet-rich plasma, PRP), je definirana kot plazemska frakcija avtologne krvi z zvišano koncentracijo trombocitov (55). Kot delovna definicija PBT je bila določena koncentracija 1,407.640/µl, kar pomeni petkratno koncentracijo trombocitov glede na kri (56).

Lastnosti PBT temeljijo na tvorbi in izločanju omenjenih bioaktivnih beljakovin, ki jih začenjo secernirati trombociti ob aktivaciji. 95% presintetiziranih beljakovin se izloči v prvi uri, nato pa jih trombociti sintetizirajo in izločajo še nekaj dni, do konca svojega življenskega cikla (55). PBT vsebuje tudi beljakovine, kot so fibrin, fibronektin, vitronektin in trombospondin. Le te so ključne pri procesih adhezije ter z njimi povezano migracijo

osteoblastov, fibroblastov in epitelnih celic. Skupni rezultat delovanja bioaktivnih beljakovin PBT je aktivacija znotrajceličnih procesov za tvorbo regenerativnih proteinov. Namen njene uporabe je torej z zvečano lokalno koncentracijo trombocitov pospešiti te, sicer fiziološke, procese. PBT je lahko pripravljena v samem operacijskem prostoru. Bolniku je odvzeta majhna količina venske krvi, iz katere lahko v nekaj minutah pridobimo PBT na tri načine: gravitacijsko sekvestracijo trombocitov, standardno separacijo krvi ter avtologno selektivno filtracijo (tromboferezo). V vseh primerih je za stabilnost PBT potrebna antikoagulantna obdelava, dekstroza in druge sestavine pa vzdržujejo metabolizem trombocitov in preprečujejo njihovo nekrozo. Praktično in koristno je, da je PBT pridobljena iz avtologne krvi, kar preprečuje nastanek avtoimunih reakcij in prenos bolezni.

Ob aktivaciji PBT s trombinom govejega ali humanega izvora nastane trombocitni gel (TG, platelet rich plasma gel, PRPG). V njem so trombociti aktivirani, kar pomeni, da izločajo iz α zrnc bioaktivne beljakovine.

Večina dostopnih sistemov priprave trombocitov uporablja podobno tehniko aplikacije (57). Gre za mešanje PBT in raztopine kalcijevega klorida s humanim trombinom v razmerju 10:1 s pomočjo dvojnega injekcijskega sistema. Tako v nekaj sekundah nastane TG, ki omogoča hipno izločanje bioaktivnih beljakovin na želenem mestu, hkrati pa zaradi viskoznosti preprečuje njihovo migracijo in omogoča njihovo tvorbo do konca življenskega cikla trombocitov.

Prve objave klinične uporabe RF kot najpomembnejše sestavine PBT segajo v l.1994, ko je bila opisana rekonstrukcija mandibule z dodatkom avtolognega fibrina in spongiozne kosti in je RTG sledenje pokazalo konsolidacijo mandibule že po 4 tednih v skupini s PBT, v skupini brez nje pa šele po 8 tednih (58). Uporaba RF, ki se izločajo iz aktiviranih trombocitov (alogenih in avtogenih), je bila dokazana tudi v stomatologiji in maksilofacialni kirurgiji (59, 60).

Prvi opisi uporabe TG na področju zdravljenja poškodb ligamentov in tetiv segajo v leto 1996 (61), nato pa so jim sledili še številni drugi (62-67).

1.4. Vloga magnetne resonance v sledenju po rekonstrukciji ACL

Magnetna resonanca (MR) je danes glavna slikovna diagnostična metoda mišičnoskeletnih obolenj. Je občutljiva (90-98%), specifična (90-100%) in točna (90-98%) metoda za ugotavljanje raztrganja sprednje križne vezi (68). MR je danes tudi standardna metoda za prikaz zapletov po rekonstrukciji ACL, kot so utesnitev in pretrganje presadka, cistična degeneracija presadka, pooperativna infekcija kolenskega sklepa, difuzna ali lokalna (oz. lezija kiklopovega očesa) artrofibroza ter spremembe na mestu odvzema (69).

Ob preizkušanju novih tehnik rekonstrukcije ACL je pomembno vprašanje ocenjevanja hitrosti vraščanja oz. zorenja presadka, saj histološke analize in meritve sile pretrganja pri ljudeh iz etičnih razlogov niso možne. Klinični testi za oceno vraščenosti presadka ACL, kot sta Lachmanov test ter sprednji predalčni fenomen, so subjektivni. Študija z artroskopijo kot zlatim standardom je pokazala značilno višjo točnost MR (86,5%) v primerjavi z artrometrom (67,3%) v oceni ohranjenosti ACL presadka (70).

MR preiskava predvsem z odlično kontrastno ločljivostjo omogoča prikaz nekaterih fizioloških procesov, kot sta edem in prekrvljenost. Za oceno edema navadno v MRI uporabljamo za tekočino občutljive pulzne sekvence, kot so T2 sekvenca, sekvenca protonske gostote (proton density, PD –), še posebno pa STIR (short tau inversion recovery) sekvenca. Z intravensko (i.v.) aplikacijo paramagnetnega kontrastnega sredstva (KS) lahko prikažemo prekrvljenost, kar danes vsakodnevno uporabljamo v klinični praksi. Še natančnejšo kvantitativno oceno edema in prekrvljenosti kot običajne sekvence omogočata kvantitativni MR preiskavi difuzije in perfuzije.

1.4.1. Difuzijsko obteženo slikanje (DWI)

Prvi opis vpliva difuzije na MR signal sega v l. 1950 (71), razvoj difuzijskega MR slikanja (diffusion weighted imaging, DWI) pa v leto 1965 (72). DWI je novejša magnetnoresonančna tehnika. Temelji na difuziji kot procesu naključnega gibanja vodnih molekul na mikroskopski ravni v tkivih in tako z izračunanim navideznim difuzijskim koeficientom (apparent diffusion coefficient, ADC) podaja kvantitativno funkcionalno oceno glede mikroskopskega gibanja vode na celični ravni. V medicini sega začetek klinične uporabe DWI v leto 1986 (73).

DWI kot tudi njena varianta traktografija sta uveljavljeni metodi v nevroradiologiji, uporabni predvsem pri odkrivanju in oceni zgodnje ishemije (74) ter drugih nevroloških okvar, kot so infekcije, tumorji in demielinizacija (75). V mišičnoskeletni radiologiji se je metoda izkazala kot uporabna pri razlikovanju med benignimi in malignimi zlomi vretenc (76), sledenju tumorjev po kemoterapiji (77) ter sledenju vnetnih lezij pri oceni zdravljenja ankilozirajočega spondilitisa (78). Kolikor nam je znano, uporaba DWI pri sledenju rekonstrukcije ACL še ni bila dokumentirana.

1.4.2. Dinamično kontrastno poudarjeno slikanje (DCEI)

Za oceno mikrocirkulacije je pri katerikoli slikovnodiagnostični metodi nujna i.v. aplikacija kontrastnega sredstva (KS).

Paramagnetna KS so organske molekule z dodanim paramagnetnim centrom. V praksi so edino razširjeno paramagnetno KS na gadoliniju (Gd) temelječi organski kompleksi. Gadolinij je sam po sebi toksičen in ga zato uporabljamo vezanega na kelat dietilen triamin pentaocetno kislino (DTPA). Le ta zaradi močnega paramagnetizma v svoji okolici izrazito spremeni magnetno polje. Zaradi tega se okoliška jedra hitreje začnejo razhajati v fazi precesije, kar

vodi do občutnega skrajšanja T2 relaksacijskega časa teh jeder. Poleg tega pa se molekule KS zaradi termičnega gibanja tudi vrtijo v spektru frekvenc, ki lahko povzročajo energijske prehode okoliških jeder in s tem do hitrejše spremembe njihove longitudinalne magnetizacije. Ta proces vodi do skrajšanja relaksacijskih časov T1 okoliških jeder. Vpliv KS na kontrast slik, posnetih z zaporedjem za MR slikanje s spinskim odmevom je sledeč: na T1 poudarjenih slikah vodi s KS povzročeno skrajšanje T1 relaksacijskega časa do povečanja signala (posvetlitev področij slike s KS), na T2 poudarjenih slikah pa vodi s KS povzročeno skrajšanje T2 relaksacijskega časa do zmanjšanja signala (potemnitev področij slike s KS). Po aplikaciji KS zato opravimo običajno le preiskavo v T1 sekvenci in jo nato primerjamo s slikami v isti sekvenci pred aplikacijo KS.

Standardna metoda MR slikanja s spinskim odmevom je precej počasna in zato ni primerna za dinamično sledenje vtoka KS. V ta namen uporabljamo hitrejše metode MR slikanja, ki temeljijo na gradientnem odmevu. Zelo je razširjena tridimenzionalna ojačana hitra metoda gradientnega odmeva (three-dimensional enhanced fast gradient echo - EFGRE3D), ki predstavlja osnovo dinamičnega kontrastno poudarjenega slikanja (dynamic contrast-enhanced imaging, DCEI). EFGRE3D metoda omogoča zajemanje številnih zaporednih slik istega reza v kratkih časovnih razmakih in tako časovni prikaz spreminjanja intenzitete signala (signal intensity, SI), t.j. perfuzije oz. mikrocikulacije, po i.v. aplikaciji paramagnetnega KS.

Kolikor smo seznanjeni, uporaba DCEI pri sledenju po rekonstrukciji ACL še ni bila dokumentirana, njen pomen pa so na področju mišičnoskeletne radiologije pokazali Reiser (79) in Ostergaard s sod. (80) s kvantitativnim ocenjevanjem vnetnih lezij pri revmatoidnem artritisu, Moehler pri oceni plazmocitoma (81) in Gašperšič (78) pri ocenjevanju aktivnosti vnetnih lezij pri ankilozirajočem spondilitisu ter njihovem odzivu na terapijo.

2. CILJI IN HIPOTEZE RAZISKAVE

2.1. Cilja raziskave

a) ugotoviti uporabnost DWI in DCEI kot dinamičnih kvantitativnih MR slikanj pri ocenjevanju vraščanja presadka ACL v tibialnem tunelu,

b) DWI in DCEI MR slikanji uporabiti kot diagnostični metodi pri ocenjevanju vloge TG pri vraščanju presadka v tibialni tunel.

2.2. Hipotezi raziskave

Zastavili smo nasledenji hipotezi:

a) DWI in DCEI kot kvantitativni MR slikanji sta uporabni diagnostični metodi za indirektno ocenjevanje edema in prekrvljenosti v tibialnem tunelu v procesu vraščanja rekonstruirane ACL.

b) z DWI in DCEI je možno neinvazivno dokazati razliko v edemu in prekrvljenosti v tibialnem tunelu pri bolnikih, ki so ob posegu lokalno prejeli TG, v primerjavi s tistimi, ki ga niso.

3. METODE

Predstavljena študija predstavlja nadaljevanje študije vpliva trombocitnega gela na vraščanje presadka križne vezi, opravljene v letih 2008 in 2009 v UKC Maribor. Izbor in randomizacija bolnikov, priprava trombocitnega gela, operativni poseg, mehanična testiranja in statistična obdelava dobljenih podatkov so bili predmet doktorske disertacije dr. Matjaža Vogrina (82) ter priloženih člankov. V sledenju bolnikov so bile MR preiskave in njihove analize opravljene s semikvantitativno, nekoliko modificirano MR metodo Gohilla s sod. (83) ter Howella s sod. in ne predstavljajo originalnega prispevka te disertacije. V pričujočem doktorskem delu, ki je bilo opravljeno na isti skupini bolnikov kot v študiji dr. Vogrina, sta predstavljeni dve novi kvantitativi metodi slikanja z magnetno resonanco: difuzijsko obteženo slikanje (DWI) in dinamično kontrastno poudarjeno slikanje (DCEI), ki po nam znanih podatkih še nista bili uporabljeni za spremljanje vraščanja po operaciji križnih vezi.

Pričujoča raziskava sledenja DWI in DCEI MRI sledenja je bila odobrena s strani Komisije za medicinsko etiko Republike Slovenije in izvedena v skladu s standardi, postavljeni s Helsinško deklaracijo iz leta 1964. Vsi bolniki so bili pred vključitvijo v študijo obveščeni o njenih ciljih, kot tudi o protokolu. Med študijo so imeli pravico biti seznanjeni z vmesnimi rezultati. Bolniki so v raziskavi sodelovali prostovoljno in to potrdili s podpisom.

3.1. Izbor preiskovancev

Vključitvena kriterija:

- simptomatsko nestabilno koleno kot posledica rupture ACL, klinično ocenjeno s strani ortopeda
- starost med 18 in 50 leti.

Izključitveni kriteriji:

- intraartikularno vnetje
- sveža poškodba (manj kot 6 tednov po njej)
- stanje po predhodni rekonstrukciji ACL
- napredovala artroza (4. stopnje po Outerbridgu)
- trombocitopenija (t<140x10/9/ml)
- ledvična insuficienca
- alergija na kontrastna sredstva
- diabetes
- maligne bolezni
- nosečnost.

3.2. Randomizacija

V raziskavo je bilo vključenih 50 bolnikov, ki so bili naključno razporejeni v dve skupini po 25 bolnikov. Bolniki v testni skupini so prejeli lokalno aplikacijo TG, medtem ko je bolniki v kontrolni skupini niso. To je bila edina razlika med skupinama.

Podatek o tem, ali je bolnik prejel TG, je vedel le operater, ne pa bolniki niti radiolog, radiološki inženirji in fizioterapevtka, ki so sodelovali pri kasnejših preiskavah in meritvah.

Vse bolnike je operiral isti specialist ortoped.

Vse kontrolne MR preiskave ter njihovo naknadno analizo je načrtoval, nadzoroval in opravil isti mišičnoskeletni radiolog.

Vse mehanične meritve je opravila ista diplomirana fizioterapevtka.

Končna odločitev o vključitvi bolnika v študijo je bila sprejeta šele po artroskopski reviziji kolenskega sklepa in oceni hrustanca. Artroskopiranih je bilo 58 bolnikov, od teh jih je bilo 8 izključenih zaradi večjih hrustančnih lezij (Outerbridge 4). Preostalih 50 bolnikov je bilo razporejenih omenjeni dve skupini s po 25 bolniki.

3.3. Predoperativna diagnostika

Delovna diagnoza raztrganine ACL je bila postavljena na podlagi anamneze, kliničnega pregleda in meritev anteroposteriorne translacije z artrometrom.

Končna diagnoza rupture ACL je bila postavljena šele pri artroskopiji, ko je bila na podlagi ocene hrustanca sprejeta tudi dokončna odločitev o vključitvi v raziskavo.

Predoperativno so bile pri bolnikih opravljene osnovne laboratorijske preiskave (KKS, CRP, krvni sladkor in kreatinin).

3.4. Operativni poseg, priprava in aplikacija trombocitnega gela ter rehabilitacija

Artroskopski poseg je bil pri vseh bolnikih opravljen s standardno operativno tehniko. Po artroskopski reviziji intraartikularnih struktur ter popravi morebitnih poškodb je bila opravljena rekonstrukcija ACL s podvojenima tetivama m. semimembranosus in m. gracilis,

ki sta bili v fiksirani v femoralni tunel z dvema prečnima žebljičkoma, v femoralnega pa z resorbilnim interferenčnim vijakom dolžine 22 mm.

Med posegom je bilo bolnikom iz testne skupine odvzete 52 ml venozne krvi. Po dodatku antikoagulantov je sledila obdelava krvi v separatorju (Medtronic, Magellan, ZDA), po kateri je bilo dobljenih 6 ml PBT. Od tega je bil 1 ml odvzet za merjenje koncentracije trombocitov. 2 ml PBT sta bila aktivirana, filtrirana in nato aplicirana s pomočjo dvojne brizgalke, kjer je prišlo do mešanja PBT in aktivnega avtolognega humanega trombina, v femoralni in tibialni tunel (po 1 ml), v katera je že bil implantiran presadek. Že nekaj sekund po takšni aktivaciji se začne tvoriti TG. Preostali 3 ml so bili infiltrirani v intraartikularni del presadka. Bolniki iz kontrolne skupine niso prejeli TG.

Pri vseh bolnikih v obeh skupinah je bila opravljena pooperativna rehabilitacija na isti način pospešenega protokola, modificiranega po Wilku (84), z začetkom takoj po operaciji. Po dvodnevni hospitalizaciji, kjer se je začela zgodnja rehabilitacija, se je le ta naslednjih šest tednov nadaljevala ambulantno. Bergle so bolniki uporabljali prvih 7-10 dni. 6-7 tednov po posegu so bolniki opravili intenzivno fizikalno rehabilitacijo v toplicah. Zatem so bolniki sami izvajali predvideni protokol vadbe. Zadnji klinični pregled so opravili 6 mesecev po posegu.

3.5. Ocenjevanje tibialnega kanala z magnetno resonanco

Kolikor smo seznanjeni, uporaba kvantitativne DWI in DCEI MR preiskave pri sledenju po rekonstrukciji ACL še ni bila dokumentirana.

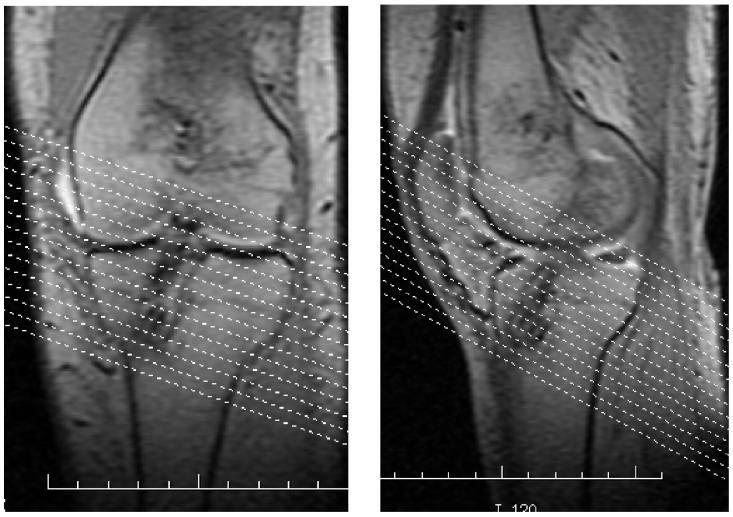
Mišičnoskeletni radiolog, ki je ocenjeval MR preiskave, ni vedel, kateri bolnik je prejel TG, bil pa je seznanjen s časom po posegu, ko je posamezni bolnik opravil kontrolno MR slikanje, saj ga je tudi organiziral. Tako je bila raziskava zanj prospektivna in dvojno slepa.

3.5.1. Protokol DWI in DCEI MR preiskave v področju presadka

Preiskave smo izvajali na MR aparatu Signa Excite 1,5 Tesla (General Electric, Waukesha, WI, ZDA) z uporabo kolenske tuljave.

Pregledovali smo tibialni tunela od platoja navzdol. Za tibialni tunel smo se odločili, ker ga je na načrtovalnih rezih preiskave lažje identificirati kot femoralnega, kar olajša načrtovanje paraaksialnih rezov. Tako DWI kot DCEI smo namreč opravljali v paraaksialni ravnini, pravokotni na potek presadka tako v koronarni kot sagitalni ravnini (slika 3).

Slika 3. Načrtovanje paraaksialnih rezov.



Komentar k sliki 3: rezi so pravokotni na os tibialnega tunela v modificiranih sagitalni in koronarni ravnini. Interesno področje (Region of interest, ROI) je zato imelo obliko kroga.

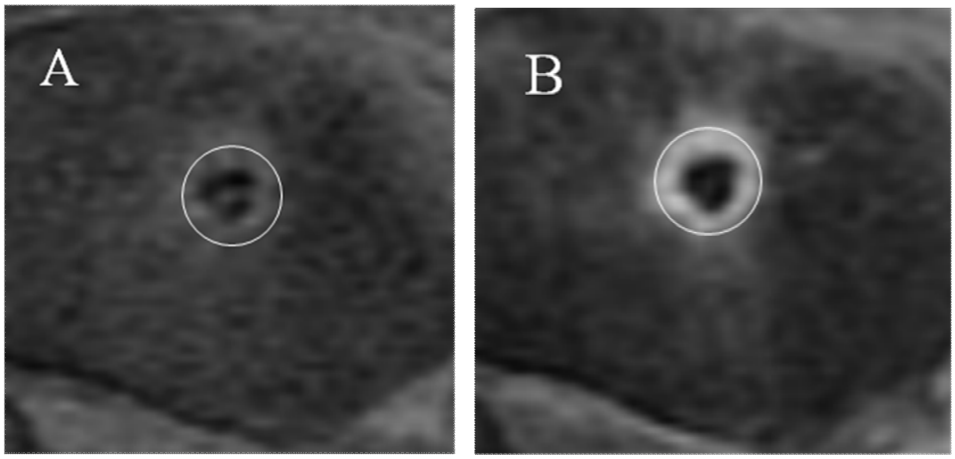
3.5.1.1. MR slikanje z difuzijsko obtežitvijo

Pred aplikacijo kontrasta smo opravili preiskavo z difuzijsko obtežitvijo (DWI). Uporabljena je bila metoda slikanja s planarnim odmevom (echo-planar imaging, EPI) z zajemom celotnega slikovnega signala po le eni sami vzbuditvi signala in pri parametrih TR=8000 ms in TE=75 ms. DWI slikanje je bilo opravljeno brez (b = 0 s/mm^2) in z difuzijsko obtežitvijo (b = 400 s/mm^2). Izbrana vrednost b = 400 s/mm^2 je predstavljala optimalno difuzijsko obtežbo ob še sprejmljivem razmerju med signalom in šumom slike. S šestnajstimi rezi je bilo pregledano področje proksimalne tibije med platojem in interferenčnim vijakom.

3.5.1.2. Dinamično kontrastno poudarjeno MR slikanje

Dinamična preiskava po aplikaciji paramagnetnega kontrastnega sredstva (DCEI) je bila opravljena z metodo EFGRE3D. Na začetku DCEI serije je bilo s črpalko (Mississipi XD 2000, Ulrich, Nemčija) s pretokom 3 ml/sekundo i.v. aplicirano paramagnetno KS Gd-DTPA (Magnevist, Schering, Nemčija) v koncentraciji 0,1 mg/kg, ki mu je sledilo še 20 ml fiziološke raztopine. Izbrano podorčje proksimalne tibije je bilo dinamično spremljano v 25 zaporednih serijah slik v 16 zaporednih prečnih rezinah, ki so bile posnete v razmaku 16 sekund (Slika 4).

Slika 4. Kvantitativna analiza perfuzije.



Komentar k sliki 4: prečni prerez skozi tibialni tunel v paraaksialni ravnini. Prva (A) in zadnja (B) slika iz niza 25 zaporednih slikanj DCEI. Na sliki je presadek hipointenziven, obdaja pa ga vmesna cona. Le ta je izrazito hiperintenzivna na sliki (B) v primerjavi z (A) kot posledica vtoka paramagnetnega kontrastnega sredstva (KS).

3.5.2. Analiza posamezne preiskave

Kvantitativno analizo slik smo opravljali v programu za obdelovanje slik (ImageJ, NIH, ZDA).

Tako pri preiskavi difuzije kot perfuzije je bil za analizo izbran prečni rez na sredini med tibialnim platojem ter proksimalnim delom interferenčnega vijaka. Na ta način smo se izognili popačitvam volumskega povprečenja s tibialnega platoja z zgornje ter interferenčnega vijaka s spodnje strani. Na izbranem rezu smo obrisovali rob tibialnega tunela, ki je predstavljal interesno področje (Region of interest, ROI). Ker gre za paraaksialno ravnino, pravokotno na os tunela, ima le-to obliko kroga, kar olajša meritve in poveča objektivnost.

3.5.2.1. Analiza slikanja z difuzijsko obtežitvijo

Pri preiskavi difuzije sta bili analizirani dve seriji slik istega področija, posneti z dvema različnima difuzijskima obtežitvama, z b vrednostima $b_0 = 0$ s/mm^2 in $b_1 = 400$ s/mm^2. SI je na DWI sliki v arbitrarnih enotah izražena z enačbo:

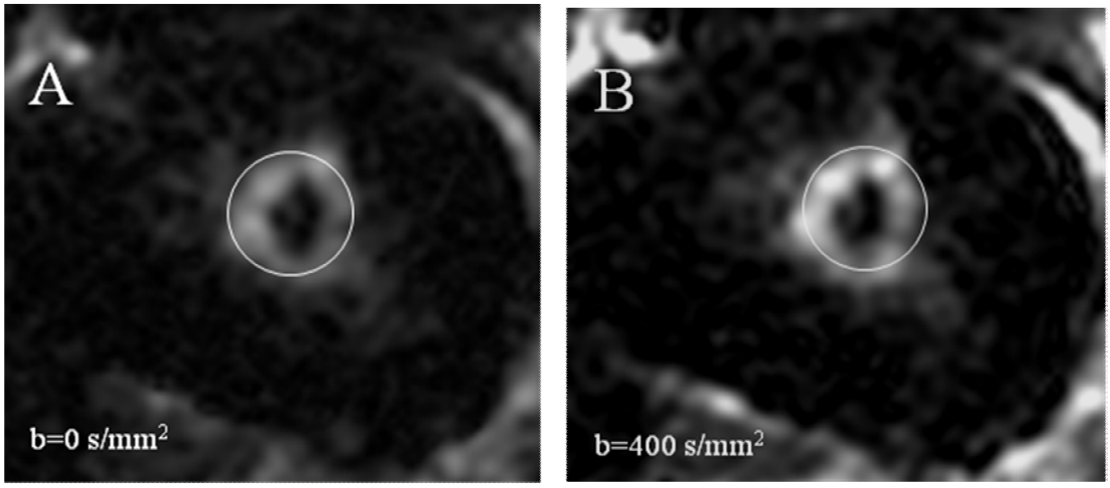
$$SI(b)=SI_0 \times \exp(-b \times ADC), \quad (1)$$

kjer je SI_0 intenziteta signala na T2 poudarjeni sliki (b=0 s/mm^2) (85). Iz zgornje enačbe smo lahko po izmerjenih SI pri dani b vrednosti za ista interesna področja izračunali vrednosti navideznega difuzijskega koeficienta ADC z uporabo formule:

$$ADC = - \ln(SI(b_1)/SI(b_0)) / (b_1 - b_0) \quad (2)$$

To smo opravili z linearno regresijsko analizo v dodatku za izračune MRI analiz programa ImageJ (ImageJ, NIH, ZDA). Tako smo dobili vrednosti ADC kot parametra za oceno prostega gibanja molekul vode v ROI (slika 5).

Slika 5. Kvantitativna analiza difuzije.



Komentar k sliki 5: za analizo smo izbrali rez na sredi med tibialnim platojem in vrhom fiksacijskega vijaka. Dve paraaksialni ADC sliki z različnima difuzijskima obtežitvima: (A) b=0 s/mm^2, (B) b=400 s/mm^2. Področja z zvišanim ADC so hiperintenzivna v primerjavi z ostalimi. Sliki sta izpeljani iz izračunov iz DWI slik po enačbi (2). V surovih DWI slikah so področja z večjo mobilnostjo vode hipointenzivna zaradi izgube signala, ki je sorazmerna difuziji. Regresijska analiza obeh slik je omogočila izračun ADC za izbrano interesno področje.

3.5.2.2. Analiza dinamičnega kontrastno poudarjenega slikanja

Preiskava omogoča meritve naraščanja intenzitete signala za izbrano ROI (slika 4).

V izbranih interesnih področjih, ki so bila ista kot za meritve difuzije, smo z uporabo istega programa, ImageJ, merili povprečne surove vrednosti intenzitete signala v odvisnosti od časa.

Dobljene vrednosti smo nato s pomočjo programa OriginPro (Origin Lab Corporation, Northampton, MA, ZDA) obdelali z matematičnim modelom po formuli:

$$SI = SI_0 + (SI_{max} - SI_0)(1-\exp(-t/\tau)) ; \quad (3)$$

kjer SI_0 (arbitrarne enote) predstavlja SI na začetku vsake DCEI serije, ki se nato eksponentno približuje največji SI_{max} v karakterističnem času τ. Šele tako smo iz vsake meritve dobili ustrezno krivuljo (slika 6).

Iz dobljenih vrednosti enačbe (3) smo izračunali gradient obarvanja (G)

$$G = 100((SI_{max}-SI_0)/(SI_0\tau)) , \quad (4)$$

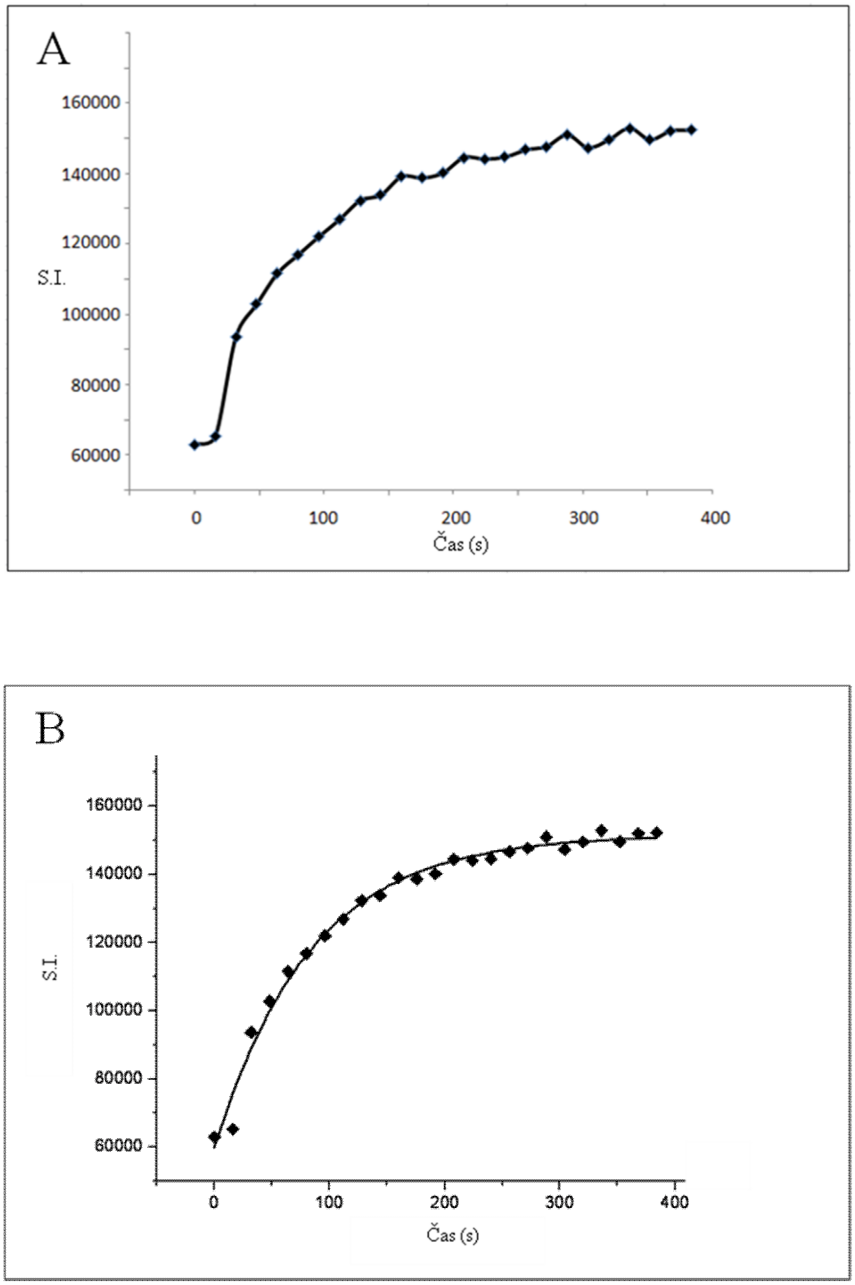
z enoto %/s, ki opisuje relativno naraščanje intenzitete signala s časom in zato predstavlja merilo za hitrost obarvanja (80).

Drug izračunan parameter je bil faktor obarvanja (F)

$$F = (SI_{max}-SI_0)/SI_0 , \quad (5)$$

ki je vrednost brez enote in predstavlja merilo za količino KS, ki se je akumuliralo v ROI glede na izhodišče in je tako merilo za statično obarvanje (80).

Slika 6. Kvantitativna analiza perfuzije.



Komentar k sliki 6: Grafa prikazujeta vrednosti intenzitete signala (S.I., arbitrarne enote) v odvisnosti od časa pri isti preiskavi. Slika (A) prikazuje surove vrednosti S.I. v odvisnosti od časa. Šele po analizi z opisanim matematičnim modelom v programu OriginPro nastane

krivulja na (B), iz katere vrednosti smo lahko izračunali parametra gradient in faktor obarvanja.

3.6. Korelacija z meritvami z artrometrom in s kliničnimi testiranji

Meritve z artrometrom in klinična testiranja je opravila ista diplomirana fizioterapevtka.

3.6.1. Ocena anteroposteriorne tanslacije tibije

Anteroposteriorno stabilnost kolena smo ocenjevali z artrometrom KT-2000 (MEDmetric, CA, ZDA) z uporabo sile 136N ter s fiksirano patelo.

Stabilnost smo ocenjevali trikrat: pred posegom ter 3 in 6 mesecev po njem. Pri vsaki kontroli smo pri vsakem bolniku opravili po tri meritve za vsako od navedenih sil, kot rezultat pa vzeli povprečje dobljenih vrednosti.

3.6.2. Ocena s funkcionalnimi vprašalniki

3.6.2.1. Tegnerjev vprašalnik

Ocena s Tagnerjevim vprašalnikom daje informacijo o največji stopnji aktivnosti. Bolniki so navedli podatke o stopnji aktivnosti pred poškodbo, pred operacijo in 6 mesecev po njej. Stopnja aktivnosti je razdeljena na 10 ravni (tabela 1):

Tabela 1: Tegnerjev vprašalnik fizične aktivnosti.

STOPNJA AKTIVNOSTI	VRSTA AKTIVNOSTI
10	Profesionalni šport - nogomet (državna raven)
9	Profesionalni šport - nogomet, košarka, gimnastika, hokej
8	Profesionalni šport – badminton, smučanje, atletika
7	Profesionalni šport: tenis, tek, rokomet Rekreativni šport: nogomet, hokej, košarka, tek, squash
6	Rekreativni šport: tenis, badminton, rokomet, smučanje, tek
5	Težko fizično delo Profesionalni šport: kolesarjenje, tek na smučeh Rekreativni šport: joging dvakrat tedensko na neravni podlagi
4	Srednje težko fizično delo
3	Lahko fizično delo
2	Zelo lahko fizično delo
1	Delo v sedečem položaju
0	Nesposobnost za delo zaradi težav s kolenom

3.6.2.2. Lysholmov vprašalnik

Lysholmov vprašalnik predstavlja subjektivno oceno stanja kolena glede na dani parameter. Bolniki so bili ocenjeni po njem pred operacijo in 6 mesecev po njej (tabela 2).

Tabela 2: Subjektivna ocena kolenske funkcije po Lysholmu.

Subjektivna ocena parametra	**Število točk**
Hoja	5-3-0
Uporaba bergel	5-2-0
Zatikanje kolena	15-10-6-2-0
Občutek stabilnosti kolena	25-20-15-5-0
Bolečina	25-20-15-5-0
Otekanje kolena	10-6-2-0
Hoja po stopnicah	10-6-2-0
Čepenje	5-4-2-0
SKUPNA OCENA	**100-0**

Komentar k tabeli 2: seštevek ocen 100 pomeni optimalno funkcijo kolena, medtem ko seštevek 0 pomeni povsem nefunkcionalno koleno.

3.6.2.3. IKDC vprašalnik

Vprašalnik IKDC je kombinacija subjektivne bolnikove ocene in objektivne ocene funkcionalnega stanja kolena.

Pri subjektivni oceni bolnik ocenjuje tri glavne kategorije, ki so nato razdeljene še v podkategorije:

- simptomi,
- raven športne aktivnosti,

- funkcija kolena.

Rezultat subjektivne ocene je seštevek točk, ki se pretvorijo v vrednost, izraženo v odstotkih, po formuli:

Vprašalnik IKDC = (seštevek ocen/največji možni seštevek ocen) x 100.

Objektivno oceno izvede fizioterapevt z oceno naslednjih parametrov:

- oteklina kolena,
- pasivna gibljivost,
- ligamentarni aparat,
- ocena funkcije medialnega, lateralnega i sprednjega kompartmenta,
- stanje mesta odvzema presadka,
- RTG izgled kolena,
- funkcionalni test (one leg hop test).

Glede na parameter se bolniki razvrstijo v tri skupine: A (normalno koleno), B (skoraj normalno koleno), C (nenormalno koleno), D (zelo nenormalno koleno). Pri razvrščanju se pri vsakem parametru upošteva najnižja ocena.

3.7. Statistična analiza

Meja statistične pomembnosti je bila pri $p<0,05$. Podatke DWI in DCEI smo obdelali s statističnim programom PASW 18 (SPSS, Chicago, IL, ZDA). Številske spremenljivke smo predstavili z aritmetično sredino in standardno deviacijo, kategorialne spremenljivke pa z deleži. Razlike v srednji vrednosti MRI meritev (ADC, G in F) med testno in kontrolno skupino smo analizirali z Mann-Whitneyevim testom. Spremembo srednjih vrednosti MRI

meritev od prve do zadnje preiskave po rekonstrukciji smo analizirali s Friedmanovo dvosmerno analizo variance. Kot post-hoc analizo smo uporabili Wilcoxonove teste predznačenih rangov, z upoštevanjem Keppelove modifikacije Bonferronijeve korekcije stopnje značilnosti alfa.

4. REZULTATI

Devet bolnikov ni opravilo vseh predvidenih kontrolnih preiskav. Po trije bolniki iz testne in iz kontrolne skupine se niso oglasili na kontrolne MR preiskave v predvidenih terminih. Pri dveh bolnikih MR preiskave zaradi tehničnih težav (napačno načrtovanje DWI in ekstravazacija kontrasta na vbodnem mestu i.v. kanala) nismo mogli opraviti. Pri enem od bolnikov je pri prvi kontrolni MR preiskavi prišlo do blažje alergične reakcije na paramagnetno KS. Vsi ti bolniki so bili zato izključeni iz raziskave. Zato smo analizirali rezultate 21 bolnikov iz testne in 20 bolnikov iz kontrolne skupine. Obe skupini preostalih bolnikov sta statistično primerljivi glede spola, starosti, strani poškodbe ter indeksa telesne mase (tabela 3).

Tabela 3. Predoperativna primerjava lastnosti med testno in kontrolno skupino.

Predoperativne lastnosti	Kontrolna skupina (N=20)	Testna skupina (N=21)	P - vrednost
Spol			0,505
moški	15	13	
ženski	5	8	
Starost (leta)	32.6±12.3	37.2±8.4	0,112
Poškodovano koleno(%)			1,000
desno	60.0	57.1	
levo	40.0	42.9	
BMI	24.5±2.1	26.5±4.1	0,078

Komentar k tabeli 3: BMI, indeks telesne mase.

4.1. Analiza PBT

Pri vseh bolnikih je bila izmerjena koncentracija trombocitov v venski krvi. Povprečna vrednost v testni skupini je znašala 190 x 10^9/l (152 do 240 10^9/l), v kontrolni pa 207 x 10^9/l (161 do 249 x 10^9/l).

Analiza PBT je pokazala visoko koncentracijo trombocitov: 978 x 10^9/l (552-1326 x 10^9/l), kar je podobna vrednost, kot opisana v literaturi (52).

4.2. MRI parametri

4.2.1. DWI

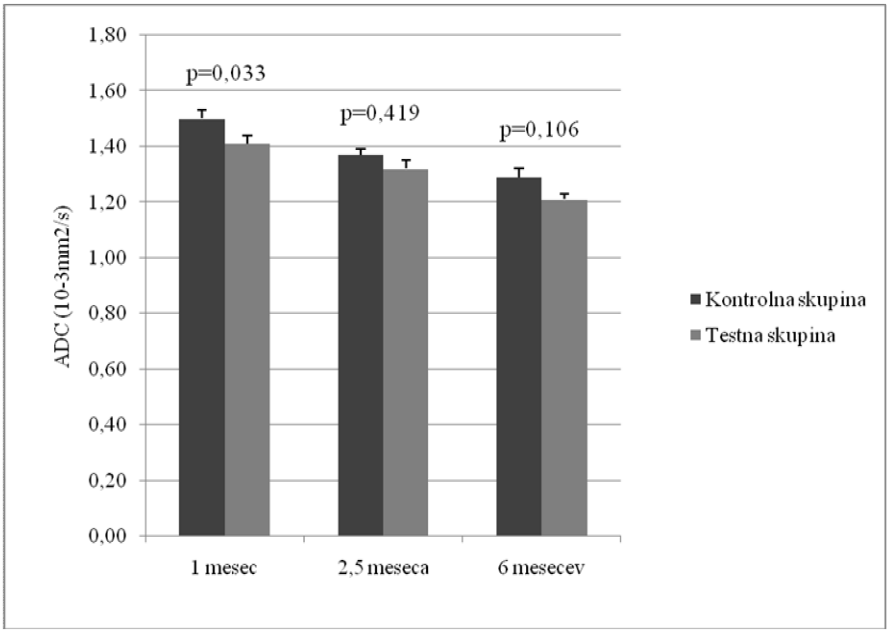
Povprečne vrednosti ADC so upadale v obeh skupinah v času sledenja (slika 7, tabela 4).

Razlike v povprečni vrednosti ADC med tremi kontrolnimi preiskavami v testni skupini so signifikantne ($p<0.001$). Post-hoc primerjave kažejo signifikantni upad povprečnega ADC s časom po posegu: 1-2 ($p=0,002$), 2-3 ($p<0,001$) in 1-3 ($p<0,001$).

Razlike v povprečni vrednosti ADC med tremi kontrolnimi preiskavami v kontrolni skupini so prav tako signifikantne ($p<0,001$). Post-hoc primerjave kažejo signifikantni upad povprečne vrednosti ADC s časom po posegu: 1-2 ($p<0,001$), 2-3 ($p=0,006$) in 1-3 ($p<0,001$).

Povprečna vrednost ADC je bila v testni skupini (1,41 ($10^{-3}mm^2/s$)) po enem mesecu značilno nižja kot v kontrolni skupini (1,50 ($10^{-3}mm^2/s$)) ($p=0,033$), kar kaže zmanjšano naključno gibanje vodnih molekul v ROI v testni skupini. Po 2,5 in 6 mesecih so bile vrednosti ADC v testni skupini le nekoliko, neznačilno, nižje kot v kontrolni skupini ($p=0,419$ in $p=0,106$).

Slika 7. Grafični prikaz primerjave povprečnih vrednosti ADC.



Komentar k sliki 7: vrednosti so podane kot povprečja (standardna napaka).

Tabela 4. Primerjava povprečnih vrednosti ADC ($10^{-3}mm^2/s$) med skupinama pri posameznih kontrolah.

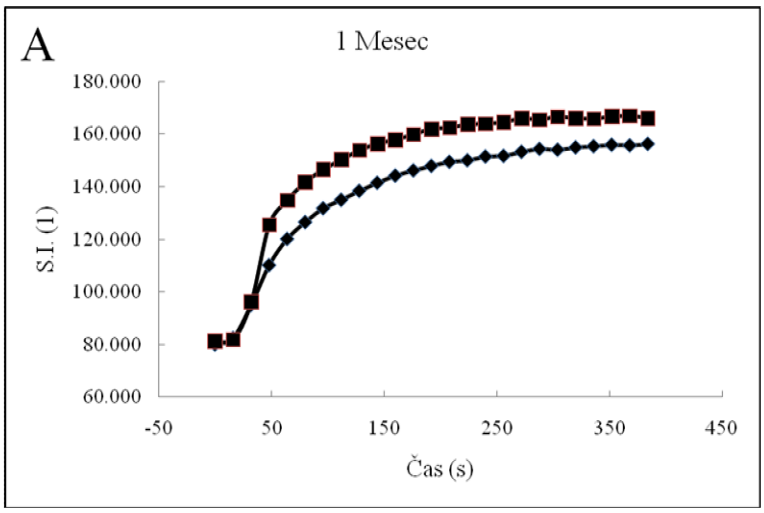
ADC ($10^{-3}mm^2/s$)	1 mesec	2,5 meseca	6 mesecev
Testna skupina	1,41* (0,03)	1,32 (0,03)	1,21 (0,02)
Kontrolna skupina	1,50* (0,03)	1,37 (0,02)	1,29 (0,03)

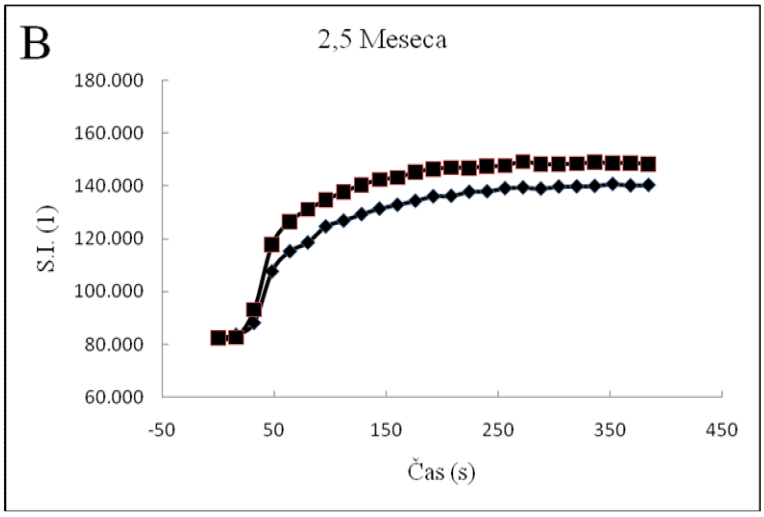
Komentar k tabeli 4: Vrednosti so podane kot povprečja (standardna napaka). *Razlika med obema skupinama je pri prvi kontroli značilna (p=0,033). ADC, navidezni difuzijski koeficient.

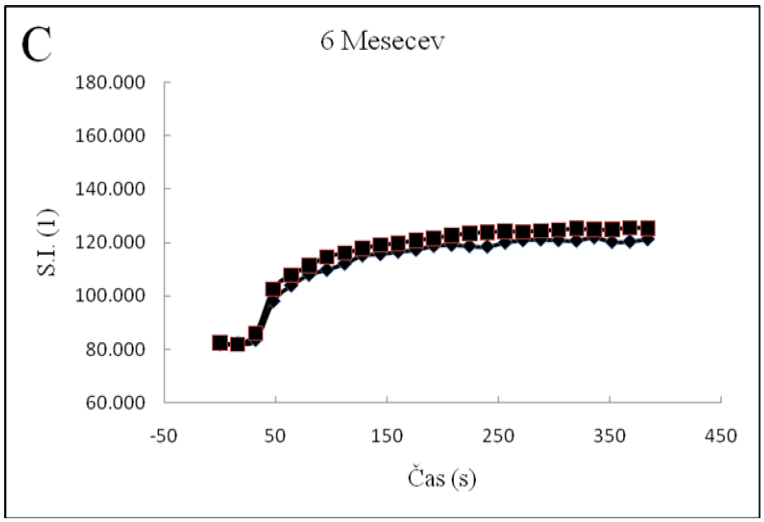
4.2.2. DCEI

Vrednosti SI so pri vsaki preiskavi najprej eksponentno naraščale in nato dosegle plato. (Slika 8).

Slika 8. Dinamična preiskava perfuzije (DCEI).







Komentar k sliki 8: Prikazane so povprečne vrednosti SI (arbitrarne enote) v interesnem področju testne (kvadrati) in kontrolne (karo) skupine v odvisnosti od časa (s) pri prvi (A), drugi (B) in tretji (C) kontroli. Pri obeh skupinah je opazno manjšanje strmine in platoja v času sledenja. Razliki med strminama krivulj pri (A) in (B) sta značilni.

Parametra F in G sta v obdobju sledenja upadala v obeh skupinah.

4.2.2.1. Gradient obarvanja (G)

Razlike v povprečni vrednosti G med tremi kontrolnimi preiskavami v testni skupini so značilne ($p<0,001$). Post-hoc primerjave kažejo signifikantni upad vrednosti G z naraščanjem časa po posegu: 1-2 ($p=0,03$), 2-3 ($p=0,001$) in 1-3 ($p<0,001$).

Razlike v povprečni vrednosti G med tremi kontrolnimi preiskavami so tudi v kontrolni skupini značilne ($p=0,007$). Post-hoc primerjave kažejo signifikantni upad vrednosti G z naraščanjem časa po posegu: 2-3 ($p=0,04$) in 1-3 ($p=0,002$). Statistično neznačilen upad smo zabeležili med prvo in drugo kontrolno preiskavo (1,41 %/s proti 1,15 %/s); p vrednost je bila blizu 0,05 ($p=0,062$).

4.2.2.2. Faktor obarvanja (F)

Razlika v povprečnih vrednostih F med tremi kontrolnimi preiskavami je v testni skupini značilna ($p<0,001$). Post-hoc primerjave kažejo signifikantni upad F z naraščanjem časa po posegu: 1-2 ($p=0,001$), 2-3 ($p=0,001$) in 1-3 ($p<0,001$).

Razlike v povprečni vrednosti F med tremi kontrolnimi preiskavami so v kontrolni skupini prav tako značilne ($p<0,001$). Post-hoc primerjave kažejo signifikantni upad vrednosti F z naraščanjem časa po posegu: 1-2 ($p=0,002$), 2-3 ($p=0,002$) in 1-3 ($p=0,001$).

4.2.2.3. Primerjava G in F med skupinama

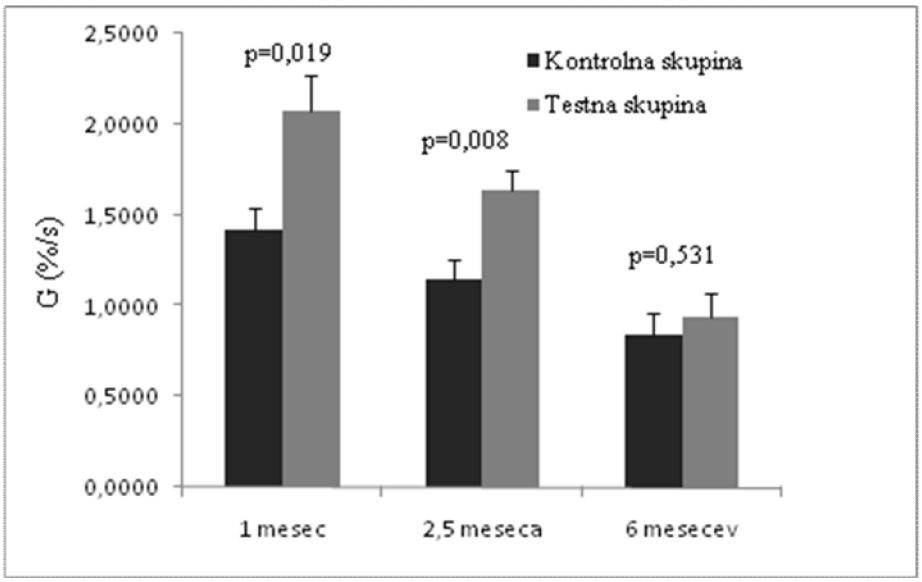
Primerjava med skupinama po enem mesecu kaže značilno višjo povprečno vrednost G v testni (2,07 %/s) skupini kot v kontrolni (1,41 %/s) skupini (p=0,019). Po dveh mesecih in pol je bila povprečna vrednost G prav tako značilno višja v testni (1,64 %/s) kot v kontrolni (1,15 %/s) skupini (p=0,008). Po 6 mesecih ni bilo signifikantne razlike med skupinama v povprečnih vrednostih G (p=0,531) (tabela 5, slika 9).

Tabela 5. Tabelarni prikaz primerjave vrednosti G (%/s).

G (%/s)	1 mesec	2,5 meseca	6 mesecev
Testna skupina	2,07* (0,20)	1,64* (0,11)	0,93 (0,13)
Kontrolna skupina	1,41* (0,13)	1,15* (0,10)	0,84 (0,12)

Komentar k tabeli 5: vrednosti so podane kot povprečja (standardna napaka). Razlika med obema skupinama je pri prvi in drugi kontroli značilna (p=0,019 in p=0,008). *p<0,05. G, gradient obarvanja.

Slika 9. Grafični prikaz primerjave povprečnih vrednosti G



Komentar k sliki 9: vrednosti so podane kot povprečja (standardna napaka).

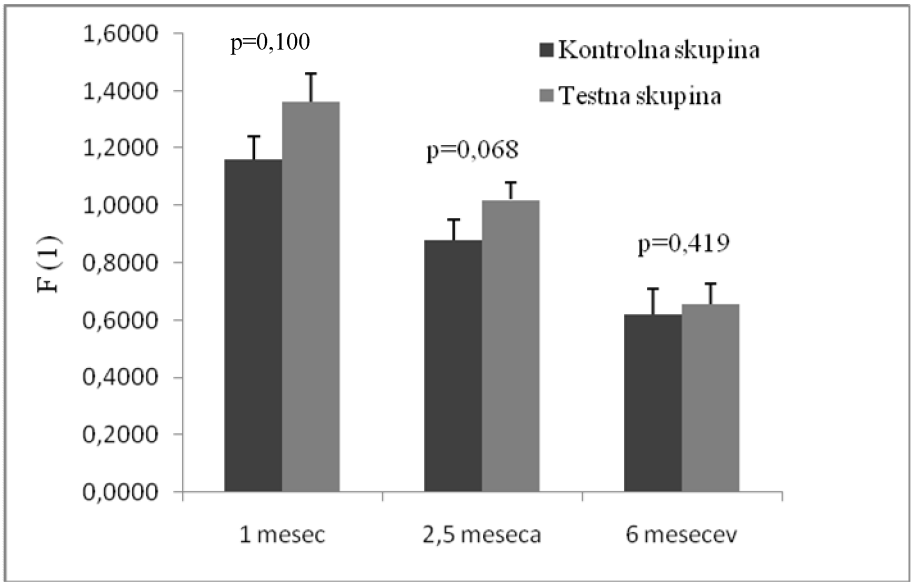
Po enem mesecu je bila tudi povprečna vrednost F v testni skupini višja, vendar ne statistično značilno (p=0,1). Tudi po dveh in pol mesecih je bila povprečna vrednost F v testni skupini višja kot v kontrolni. Razlika ni bila značilna, vendar pa je bila p vrednost blizu 0,05 (p=0,068). Po šestih mesecih med skupinama ni bilo razlike v povprečnih vrednostih F (p=0,419) (tabela 6, slika 10).

Tabela 6. Tabelarni prikaz primerjave vrednosti F.

F (1)	1 mesec	2,5 meseca	6 mesecev
Testna skupina	1,36 (0,10)	1,02 (0,06)	0,66 (0,07)
Kontrolna skupina	1,16 (0,08)	0,88 (0,07)	0,62 (0,09)

Komentar k tabeli 6: Vrednosti so podane kot povprečja (standardna napaka). F, faktor obarvanja.

Slika 10. Grafični prikaz primerjave povprečnih vrednosti F



Komentar k sliki 10: vrednosti so podane kot povprečja (standardna napaka).

4.3. Analiza AP stabilnosti in funkcionalni vprašalniki

4.3.1. Analiza z artrometrom

Meritve z artrometrom KT-2000 pred posegom so pokazale praktično identično stabilnost testne in kontrolne skupine.

Meritve tri in šest mesecev po operaciji so pokazale značilno večjo stabilnost v testni skupini v primerjavi s kontrolno (tabela 7).

Tabela 7. Analiza anteroposteriorne translacije tibije.

A-P translacija tibije (mm)	Kontrolna skupina (N=20)	Testna skupina (N=21)	P-vrednost
KT-2000 vrednost pred posegom (136 N)	7,9±2,7	7,9±2,9	0,925
KT-2000 vrednost 3 m po posegu (136 N)	6,1±2,1	4,9±1,8	0,035
KT-2000 vrednost 6 m po posegu (136 N)	6,7±2,1	4,7±1,9	0,003

Komentar k tabeli 7: vrednosti so podane so kot povprečja (standardna napaka). Meritve kažejo značilno manjšo translacijo v testni skupini po 3 in 6 mesecih.

4.4. Klinična testiranja

4.4.1. Tegnerjev vprašalnik

Primerjali smo rezultate ankete pred poškodbo, tik pred posegom in 6 mesecev po njem (tabela 8).

Tabela 8. Analiza stopnje aktivnosti po Tegnerju.

Tegnerjev vprašalnik (0-10)	Kontrolna skupina (N=20)	Testna skupina (N=21)	P-vrednost
Pred poškodbo	6,75±1,33	6,67±1,39	0,84
Pred posegom	3,45±1,57	3,27±1,31	0,71
6 mesecev po posegu	5,25±1,68	5,57±1,78	0,55

Komentar k tabeli 8: vrednosti so podane so kot povprečja (standardna napaka).

Rezultati kažejo relativno veliko stopnjo aktivnosti pred poškodbo, 6,67 v testni in 6,75 v kontrolni skupini. Po poškodbi je prišlo do velikega upada aktivnosti na 3,27 v testni in 3,45 v kontrolni skupini. 6 mesecev po posegu je aktivnost pričakovano narastla na 5,57 v testni in 5,25 v kontrolni skupini. Razlika med skupinama ni bila značilna.

4.4.2. Lysholmov vprašalnik

Bolniki so bili s funkcionalnim vprašalnikom po Lysholmu ocenjeni pred posegom in 6 mesecev po njem (tabela 9).

Tabela 9. analiza funkcije kolena po Lysholmu.

Lysholm vrednost (0-100)	Kontrolna skupina (B=20)	Testna skupina (B=21)	P-vrednost
Pred posegom	73,40±16,46	65,29±18,74	0,15
6 mesecev po posegu	91,70±10,42	89,86±10,53	0,576

Komentar k tabeli 9: vrednosti so podane so kot povprečja (standardna napaka).

Pred posegom so bile vrednosti ankete nizke, in sicer 65,29 v testni in 73,40 v kontrolni skupini. Rezultati potrjujejo pričakovan porast vrednosti vprašalnika 6 mesecev po posegu na 89,96 v testni in 91,70 v kontrolni skupini. Razlika med skupinama ni bila značilna.

4.4.3. IKDC vprašalnik

Funkcionalni vprašalniki kolenskega sklepa po IKDC so sestavljeni tako iz objektivnih kliničnih meritev kot subjektivnih bolnikovih ocen. Bolniki so bili ocenjeni pred posegom in 6 mesecev po njem (tabela 10).

Tabela 10. Analiza funkcionalnih testov po IKDC.

IKDC vrednost (0-100)	Kontrolna skupina (B=20)	Testna skupina (B=21)	P-vrednost
Pred posegom	54.54±18.09	49.92±16.44	0.4
6 mesecev po posegu	76.62±9.96	77.60±10.82	0.765

Komentar k tabeli 10: vrednosti so podane so kot povprečja (standardna napaka).

Pred posegom so imeli bolniki nizke vrednosti tako v testni (49,92) kot kontrolni (54,54) skupini. Po posegu so vrednosti pričakovano narasle na 77,60 v testni in 76,62 v kontrolni skupini. Razlika med skupinama ni bila značilna.

5. RAZPRAVA

V zadnjih desetletjih smo priča številnim novostim rekonstrukcije ACL. Ker histološka ocena vraščanja pri ljudeh ni možna, ima pomembno vlogo pri ocenjevanju modifikacij rekonstrukcije MR, zato njena uporaba v tem področju narašča.

Proces zorenja presadka so z MR prvič spremljali l. 1991 (86, 87). Študije, ki so sledile, so bile usmerjene predvsem v ocenjevanje intraartikularnega zorenja presadka oz. ligamentizacije (70, 88-90), dolžine presadka (91), mesta odvzetih tetiv za rekonstrukcijo (92), delno tudi v resorpcijo fiksacijskega vijaka (93), merjenje širine tibialnega tunela (91, 94, 95) in edema ob njem (93), manj pa v vraščanje ligamenta v sam tunel (87, 90, 92, 94,

96). Slednje pa je pri ocenjevanju učinka različnih RF, apliciranih v tibialni tunel ter presadek v njem, najpomembnejše.

Kolikor nam je znano, je prva študija ocenjevanja učinka lokalne aplikacije TG z MR bila objavljena l. 2008. Orrego s sod. (51) je za razliko od naše študije ocenjeval femoralni tunel ter homogenost signala celotnega presadka, prisotnost vmesne cone med presadkom in steno tunela in premer tunela. Po treh mesecih po rekonstrukciji pri nobenem od MR kriterijev ni našel razlik med skupinama. Po 6 mesecih je v testni skupini našel hipointenziven signal presadka pri vseh bolnikih v primerjavi z le 78% presadkov kontrolne skupine, razlika je bila značilna. Študijo zaradi skoraj povsem drugačne metodologije težko primerjamo z našo, menimo pa, da so takšni rezultati vsaj delno rezultat ocenjevanja z metodo vizualnih opažanj, ki so subjektivna, predvsem pa manj občutljiva kot izračuni kvantitativne MR. Pri naši raziskavi je bilo subjektivno le obrisovanje tibialnega tunela, pa še to smo omejili s paraaksialnimi rezi, tako da je imel prečni prerez vedno obliko kroga. Glavno prednost naše raziskave pa vidimo v tem, da smo ocenjevali tibialni tunel v zgodnji fazi po rekonstrukciji, kar so potrdili tudi rezultati.

Nin s sod. (52) je za razliko od naše študije opravljal rekonstrukcijo z ligamentom patele. Šest mesecev po posegu je z MR ocenjeval vpliv TG na debelino, intenziteto in homogenost signala presadka intraartikularno in v tibialnem tunelu, ob tem pa tudi anteriorno translacijo tibije in položaj zadnje križne vezi. Pri tem ni našel razlike med skupinama niti v omenjenih MR parametrih niti v kliničnih in funkcionalnih testih. Tudi Figueroa (54) 6 mesecev po rekonstrukciji s presadkom iz tetiv hamstringov z MR ni našel razlike v signalu presadka niti vmesne cone med skupino, ki je lokalno prejela TG, in kontrolno skupino. Rezultati obeh študij se skladajo z našimi, ko v tem času prav tako nismo našli razlik v MR parametrih. Menimo, da so takšni rezultati po 6 mesecih razumljivi, saj je v tem času proces vraščanja že

napredoval. Zato sodimo, da sklep študij Nina in Figueroe s sod., da PBT nima učinka, temelji na prepoznem času sledenja.

Radice s sod. (53) je z MR prav tako vizualno ocenjeval vpliv lokalne aplikacije TG na homogenost presadka. Ugotovil je, da so presadki v testni skupini značilno prej, že po 6 mesecih, pridobili homogen signal v primerjavi s kontrolno, kjer je bil signal homogen šele po enem letu. Pri tej študiji so uporabljali različne presadke glede na vrsto aktivnosti ob poškodbi, prav tako pa so testno skupino ocenjevali od tretjega meseca po rekonstrukciji naprej, kontrolno pa šele od šestega. V tem se študija bistveno razlikuje od naše, poleg tega pa tudi v tipu presadka in usmerjenosti avtorjev v intraartikularni del presadka.

Če strnemo, nobena od naštetih študij ni ocenjevala bolnikov v prvih dveh mesecih in pol, torej v zgodnjem obdobju, v katerem so histološke študije pokazale največji učinek RF (13, 18, 45). Pri vseh so uporabljali kvalitativne (51-54) ali semikvantitativne (52 – intenziteta signala) oblike vizualnega ocenjevanja s prostim očesom. Nobena ni ocenjevala prekrvljenosti z i.v. aplikacijo paramagnetnega KS. Še več, našli smo celo študijo, ki govori o oceni vaskularizacije, ne da bi bolnikom sploh aplicirali KS (97).

Uporabnost obeh kvantitativnih MR metod, DWI in DCEI, v sledenju tibialnega tunela po rekonstrukciji ACL, smo pokazali v posebni raziskavi (98).

MR slikanje z difuzijsko obtežitvijo omogoča z merjenjem difuzije vodnih molekul natančnejšo kvantitativno oceno mobilnosti vodnih molekul oz. edema v ekstracelularnem prostoru kot standardne, za tekočino občutljive MR sekvence.

Molekula se s časom oddalji od njene začetne lege v naključni smeri. Pri telesni temperaturi molekule vode naključno migrirajo za pribl. 30 μm v 50 ms, če njihovo gibanje ni ovirano (99). Z uporabo bipolarnih gradientnih sunkov, dodanih slikanju v T2 sekvenci, lahko zaznamo njihove premike. Vsaka molekula bo namreč po končanem takšnem sunku pridobila fazo, ki bo odvisna od njenega premika. Zaradi različnih premikov različnih molekul se

pridobljene faze med seboj razlikujejo. Zato se zaradi različnih faz signalov celotni signal po bipolarnem gradientnem sunku zmanjša, podobno kot se to zaradi enakega razloga zgodi pri T2 relaksaciji, njegova faza pa se ne spremeni. Zmanjšanje signala je večje pri živahnejšem gibanju molekul, odvisno pa je tudi od jakosti in trajanja bipolarnih gradientnih sunkov. Slednje označujemo kot faktor občutljivosti difuzije oz. b vrednost (74). Po Fickovem zakonu je prava difuzija neto gibanje molekul zaradi koncentracijskega gradienta. Z MR ne moremo razlikovati med gibanjem molekul zaradi koncentracijskega, tlačnega in temperaturnega gradienta ter zaradi ionskih interakcij. Zato lahko pri merjenju z DWI iz izmerjene SI pri dani b vrednosti izračunamo le navidezni difuzijski koeficient ADC (99).

Difuzijsko preiskavo navadno opravimo z ehoplanarnim slikanjem brez (b=0 s/mm^2) in z difuzijsko obtežitvijo (b=100-1000 s/mm^2). Na osnovi merjenja zmanjšanja signala lahko tako izmerimo tudi difuzijsko konstanto snovi. Ker so premiki molekul pri difuziji dosti manjši kot pri pretakanju krvi, moramo za njeno merjenje uporabiti tudi precej močnejše in dolgotrajnejše bipolarne gradientne sunke, kot so potrebni za merjenje pretoka. V primerjavi z difuzijsko MR preiskavo z nizkimi b vrednostmi (50-100 s/mm^2), ko na vrednost ADC vplivata tako mikrocirkulacija kot vsebnost in mobilnost vode v zunajceličnem prostoru, pri višjih b vrednostih vpliva mikrocirkulacije praktično ni več (99). Zato vrednosti ADC v naši študiji, izračunane iz podatkov, zajetih ob parametru b=400 s/mm^2, realneje odražajo mobilnost vode v zunajceličnem prostoru kot pri nižjih vrednostih b.

V tkivih gibanje vode ni niti povsem prosto niti naključno. Nanj namreč vplivajo interakcije z celičnimi membranami, organeli, makromolekulami in pretok v okolnih tubularnih kanalih, kot so žile, mezgovnice in drugi vodi. Molekule zunajcelične vode se zato lažje gibljejo v primerjavi z znotrajceličnimi. Difuzija vode pri klinični uporabi DWI zato odraža lastnosti tkivne organizacije, predvsem število celic v volumski enoti tkiva. Področja z zvečanim številom celic in zato več znotrajceličnimi komponentami in membranami bolj ovirajo

mobilnost vode kot področja z izgubo celične integritete, kot npr. nekroza. DWI tako prispeva funkcionalno informacijo na celični ravni pri razlikovanju med normalnim in patološkim tkivom (99).

Na ADC slikah (slika 5), kjer je vrednost ADC izračunana iz enačbe (2) in svetlost sorazmerna vrednosti ADC, je bila v naši študiji vmesna cona v tibialnem tunelu pri vseh preiskavah, najbolj pa po enem mesecu, v obeh skupinah bolnikov vizualno hiperintenzivna v primerjavi z okolno kostnino ter podkožnim maščevjem. Ta videz je bil najizrazitejši pri prvi kontroli v primerjavi z naslednjima, kar je v skladu z manjšanjem mobilnosti vode v procesu vraščanja in kar so potrdili tudi izračuni ADC. Le ti omogočajo kvantitativno oceno teh opažanj.

Izračunane vrednosti ADC v interesnem področju so bile pri vseh preiskavah višje kot v kostnem mozgu (povprečje ADC = 0.15 x 10^{-3} mm^2/s (98) do 0.23 x 10^{-3} mm^2/s (100) in nižje kot vrednosti proste vode (povprečje ADC = 2.80 x 10^{-3} mm^2/s (101). Povprečne vrednosti ADC so v obdobju sledenja med vsemi kontrolnimi preiskavami značilno upadale v obeh skupinah, kar je dokaz manjšanja mobilnosti vode v tibialnem tunelu ob procesu vraščanja. To se sklada z že v uvodu opisanimi histološkimi opisi vraščanja presadka, ki vključujejo množenje nevtrofilcev, makrofagov in fibroblastov v procesu, ki se konča s tvorbo fibroznega in končno kostnega tkiva. (18, 24, 25). Najdba potrjuje prvo hipotezo uporabnosti DWI preiskave v pooperativnem sledenju vraščanja ACL presadka.

Značilno nižje vrednosti ADC v interesnem področju v testni skupini en mesec po rekonstrukciji odražajo manjšo mobilnost vode v ekstracelularnem prostoru, torej edema, v tibialnem tunelu. Nižje vrednosti ADC najdemo v področjih z zvečanim številom celic, kot je npr. kostni mozeg, zajet z osteomielitisom (103) ali metastazami (104-106). Gre za procese, kjer je mobilnost vode zaradi infiltracije z vnetnim ali tumorskim tkivom manjša (99). Zato se značilno nižje vrednosti ADC v naši študiji skladajo s histološko dokazanim večjim številom

kolagenskih fibril (45) in fibroblastov (47) kot učinkom PDGF (45) in VEGF (47). Rezultat je skladen tudi z večjo gostoto prečnih kolagenskih vlaken v osteoligamentozni vmesni coni kot učinkom lokalne aplikacije TGF-ß1 (46) ter hrustanca in kostnine v isti coni pri študijah, kjer so uporabili gensko terapijo kot način vnosa in podaljševanje učinka RF (43, 44) ter pri lokalni aplikaciji BMP-2 (42) in BMP-7 (48).

Ocena prekrvljenosti pri MR temelji na zvišanju SI v T1 sekvenci po i.v.aplikaciji paramagnetnega KS kot posledica skrajšanja relaksacijskega časa s kontrastom infiltriranega tkiva. Kvalitativno so prekrvljenost po rekonstrukciji ACL v vmesni coni ter samem presadku z i.v. aplikacijo paramagnetnega KS ocenjevali prvič leta 1995 (90). Ocena je tako vizualna, možna pa je tudi semikvantitativna ocena z merjenjem SI v interesnem področju v arbitrarnih enotah. Razlika med SI pred in po aplikaciji KS, t.j. jakost obarvanja, predstavlja merilo statične prekrvljenosti, sorazmerne velikosti intravaskularnega ter intersticijskega prostora in prehajanju KS med njima (81).

DCEI kot kvantitativna metoda omogoča natančnejšo oceno prekrvljenosti, saj omogoča oceno kinetike razporeditve paramagnetnega KS v mikrocirkulaciji ter intersticiju preiskovanega tkiva, ki oba pripadata ekstracelularnem kompartmentu, in vivo. Še več, ker nove metode rekonstrukcije ACL praviloma izzovejo večjo prekrvljenost (45), je njena kvantitativna ocena toliko pomembnejša. Časovna sprememba signala po i.v. aplikaciji KS je posledica kompleksnega procesa. Gadolinijeva KS so namreč ekstracelularna KS, katerih razporeditev je povezana z lokalno prekrvljenostjo ter prehajanjem KS v intersticijski prostor, razen v centralnem živčevju, saj ne prehajajo normalne hematoencefalne bariere (107, 108). Beležene podatke lahko grafično prikažemo s krivuljo SI v odvisnosti od časa (slika 6). Iz slednje najpogosteje izluščimo dva podatka: a) strmino (parameter G), ki označuje hitrost obarvanja in s tem hitrost vtoka KS in je torej odvisna predvsem od velikosti intravaskularnega kompartmenta, in b) plato (parameter F), ki označuje jakost obarvanja in je

odvisna tudi od hitrosti prehajanja KS iz intravaskularnega v intersticijski prostor (81, 107). Oba parametra odražata značilnosti mikrocirkulacije, kot so perfuzija, volumen intravaskularnega prostora in permeabilnost kapilar (107). Ocena mikrocirkulacije je pomembna pri procesih z zvečano prekrvljenostjo, t.j. hiperemijo. To so predvsem tumorji in vnetja. Pri številnih tumorjih je gostota krvnih žil sorazmerna tako njihovi lokalni invazivnosti kot tudi hitrosti zasevanja. Med njimi so najpogostejši karcinomi dojke, pljuč in prostate (108). Tumorji namreč ne tvorijo lastnih krvnih žil, ampak so odvisni od žil iz soseščine, po katerih prejemajo hrano in kisik. Tumorske celice izločajo stimulatorje angiogeneze, ki delujejo lokalno parakrino s stimulacijo tvorbe endotelijskih celic in vraščanja kapilar iz okolice. Po stimulaciji angiogeneze zato tumorji preidejo v t.i. žilno fazo, v kateri rastejo eksponentno in se začno klinično manifestirati. Med stimulatorje tumorske angiogeneze spadajo nekateri med že v uvodu opisanimi RF, predvsem pa VEGF (109). Zato so za zdravljenje med drugim v uporabi tudi protitelesa proti tem RF (110). DCEI je zaradi specifične tumorske mikrocirkulacije zato uporabna metoda pri odkrivanju, zamejitvi in odzivu na zdravljenje, npr. karcinoma prostate (111). Uporaba DCEI je bila sicer opisana tudi v oceni plazmocitomskih infiltratov (81) ter tumorjev obraza in vratu (112).

Tudi pri vnetnih procesih igrajo različni RF pomembno vlogo, npr. PDGF, ki pri sinovitisu na isti način povzročajo povečano prekrvljenost (113). Zato je DCEI MR preiskava uveljavljena metoda za oceno aktivnosti sinovitisa že od 1989 (79), opis korelacije najdb DCEI s histološkimi pa sega v l. 1998 (80). DCEI je bila tako uporabljena tudi za oceno vnetnih sprememb sakroiliakalnih sklepov in odziva na zdravljenje pri ankilozirajočem spondilitisu (78).

Vrednosti SI pri DCEI v interesnem področju so po i.v. aplikaciji KS pri vsaki preiskavi zaradi arterijskega vtoka hitro narasle in dosegle plato ob prehajanju v intersticijski prostor. V času opazovanja (400 s) nismo zasledili izplavljanja KS iz interesnega področja.

Povprečne vrednosti DCEI parametrov, gradienta (G) in faktorja (F) obarvanja, so v naši študiji v obdobju sledenja med vsemi kontrolnimi preiskavami značilno upadale v obeh skupinah, kar je v skladu s histološkimi opisi zmanjševanja prekrvljenosti kot del manjšanja vnetnega odgovora in brazgotinjenja v procesu vraščanja presadka (18, 24, 25). Ta rezultat potrjuje prvo hipotezo uporabnosti DCEI preiskave v pooperativnem sledenju vraščanja ACL presadka.

Gradient obarvanja G predstavlja strmino krivulje SI-čas, torej merilo za hitrost obarvanja ob. vtoku KS (107). Le ta je povečana pri vnetnih (80) in tumorskih (112, 114) procesih, kjer igrajo RF prav tako pomembno vlogo in kjer predstavljata oba parametra, G in F, pomembna označevalca angiogeneze (107, 109).

Značilno višjo povprečno vrednost G in torej večjo hitrost vtoka KS v testni skupini pri prvi in drugi kontrolni preiskavi pripisujemo večjemu intravaskularnemu kompartmentu, torej volumnu žil, v tibialnem tunelu. Rezultat je skladen s histološkimi opisi večje gostote krvnih žil v živalskih presadkih po lokalni aplikaciji PDGF (45) ali VEGF (47, 50) 3-12 tednov po rekonstrukciji. Le neznačilno višjo vrednost G v testni skupini po 6 mesecih bi lahko razložili s hitrejšim upadom prekrvljenosti, najdenim histološko v poznih fazah pri zajcih, ki so prejeli VEGF, kar bi lahko bila posledica zgodnejšega zorenja presadka (50), poleg tega pa tudi z manjšo natančnostjo meritev pri tako majhnih vrednostih.

Parameter F, predstavljajoč relativno obarvanje v interesnem področju in torej plato krivulje SI-čas (80, 107), je tesno povezan z velikostjo ekstracelularnega prostora, ki se izpolni s KS po njegovi i.v. aplikaciji in vsebuje tako intravaskularni kot tudi intersticijski prostor (81). Višina tega platoja je tako odvisna od gostote krvnih žil, poleg tega pa tudi od njihove permeabilnosti (107, 115), ki vpliva na hitrost izmenjave med obema kompartmentoma. Medtem ko se dinamiki vrednosti G in F navadno skladata (107, 109, 115), sta le neznačilno višji vrednosti F v testni skupini pri prvi in drugi kontroli za razliko od značilno višjih

vrednosti parametra G v istem času nekoliko nepričakovani. Lahko gre le za posledico relativno majhnega vzorca bolnikov, saj p vrednosti pri razlikah parametrov F med skupinama nista visoki (0,100 pri prvi kontroli in 0,068 pri drugi). Zato bi bilo treba tak rezultat preveriti v raziskavi z večjim številom bolnikov. Po drugi strani pa le neznačilno povečanje parametra F v testni skupini nakazuje možnost, da je parameter G še občutljivejši za oceno prekrvljenosti kot F, saj odraža predvsem gostoto krvnih žil, F pa skupno kopičenje KS v ekstracelularnem prostoru, ki vključuje tako intravaskularni kot intersticijski prostor in je torej odvisno tako od volumna žil kot tudi hitrosti izmenjave med obema kompartmentoma. Ker so rezultati DWI nakazali večje število celic v ROI in torej manj intersticija, gre povečanje faktorja F pripisati povečanju tako intravaskularnega volumna kot tudi permeabilnosti žil. Takšna najdba bi bila v skladu z navedbami Ostergaarda (80), ki je našel visoko korelacijo laboratorijskih, makroskopskih in mikroskopskih znakov vnetja pri revmatoidnem sinovitisu s hitrostjo, ne pa tudi stopnjo statičnega obarvanja.

V vsakem primeru značilne razlike med skupinama v vrednostih ADC po enem mesecu in G po enem in dveh in pol mesecih v naši študiji potrjujejo zgodnje učinkovanje lokalno apliciranega TG na proces vraščanja presadka, kar je tudi v skladu s histološko dokazanimi zgodnjimi učinki RF in drugih novejših tehnik rekonstrukcije ACL (45-47). Ti rezultati potrjujejo drugo hipotezo naše študije o dokazu razlik v kvantitativnih MR parametrih pri oceni edema in prekrvljenosti kot učinkov TG.

6 mesecev po posegu z nobenim od parametrov kvantitativne MR preiskave nismo našli značilne razlike med skupinama. To pojasnjujemo s tem, da so vrednosti MR parametrov, ki odražajo edem in prekrvljenost, v tem času že tako nizke, da z njimi nismo mogli več zaznati razlik med skupinama. Zgodnji procesi vraščanja so v tem času že praktično končani, v ospredju pa je kostno preraščanje vmesne cone, ki se odraža v stabilnosti sklepa, ki jo lahko izmerimo.

Meritve z artrometrom so pred posegom v obeh skupinah pokazale praktično enako nestabilnost. V obeh skupinah smo po posegu izmerili zmanjšanje anteroposteriorne translacije. Značilno nižja izmerjena AP translacija v testni skupini v primerjavi s kontrolno po treh in šestih mesecih najverjetneje predstavlja posledico hitrejšega vraščanja in je v skladu z najdbo večje stabilnosti oz. sile pretrganja presadka pri histoloških študijah, kjer so uporabljali različne RF (42-46, 49, 116). Po treh mesecih smo pri MR meritvah zabeležili le značilno večji gradient obarvanja kot indirektnega pokazatelja večje gostote krvnih žil z že zgoraj opisano histološko korelacijo. Protokol študije ni predvideval testiranja z artrometrom prvi mesec po posegu, ko je bila razlika v MR parametrih med obema skupinama največja. Zato predlagamo, da naj bodoče podobne študije predvidevajo takšna testiranja tudi v zgodnejšem obdobju.

Analiza s funkcionalnimi vprašalniki je bila opravljena pred posegom in 6 mesecev po njem. Pri vseh vprašalnikih je prišlo do porasta vrednosti stopnje fizične aktivnosti ter porasta vrednosti kliničnih meritev in subjektivnih ocen bolnikov v obeh skupinah po 6 mesecih v primerjavi s predoperativnimi testiranji. Vrednosti se med skupinama niso značilno razlikovale. To pripisujemo dejstvu, da gre ima pri teh vprašalnikih pomembno vlogo fizična aktivnost, ki pa je glede na protokol rehabilitacije, ki je bil uporabljen, morala biti v tem času še omejena. Zato razlike med skupinama niti ni bilo za pričakovati (82).

6. OMEJITVE RAZISKAVE

Iz etičnih razlogov naših najdb ni možno primerjati s histološkimi.

ROI je v naši študiji obsegal celoten tibialni tunel, kar pomeni tako vmesno cono kot tudi sam presadek, kar predstavlja možno omejitev študije. Ločena analiza vmesne cone bi lahko bila natančnejša; po drugi strani pa bi bila zaradi omejenega števila pikslov v ROI ponovljivost

meritev manjša. Pri nobeni preiskavi sicer nismo opazili vidne razlike v intenziteti signala presadka v ROI. Ob tem tudi v histoloških opisih vraščanja v tunel nismo našli opisov vaskularizacije samega presadka. Večjo točnost bi dosegli,če bi opravili MR meritve v več rezih tunela med platojem in vijakom ter nato izračunali povprečne vrednosti parametrov, vendar bi takšen postopek predstavljal časovni večkratnik opravljenih meritev ter izračunov, ki so že tako bili dolgotrajni. Primerjava z rezultati histoloških študij pa kaže, da lahko tudi na podlagi le enega analiziranega reza sklepamo na dogajanje v celotnem tunelu. Večjo natančnost bi dosegli tudi s primerjanjem rezultatov več ocenjevalcev, čeprav smo se subjektivnosti obrisovanja tibialnega tunela v največji možni meri izognili s preiskavo v paraaksialnih rezih, pravokotnih na tunel, ko je njegov prečni presek predstavljal krog. Intraartikularnega dela presadka ter presadka v femoralnem tunelu nismo ocenjevali zaradi tehničnih omejitev, kot je omejeno število rezov pri DWI in toplote, nastale z energijo ponavljajočih RF sunkov pri DCEI, ki bi pri večjem številu rezov, če bi zajemali tudi femoralni kanal, bila zelo velika. Poleg tega nismo izvedli predoperativnih MRI preiskav pri vseh bolnikih, kot tudi nismo ocenjevali vpliva stopnje artroze oz. učinka morebitnih pridruženih rekonstruktivnih posegov pri artroskopiji, kot tudi ne stopnje kroničnosti, kar bi vse lahko prispevalo k vnetnemu odgovoru, čeprav je le-to vprašljivo v kanalu v primerjavi z intraartikularnim vnetjem. Število bolnikov v študiji je omejeno, v glavnem zaradi zapletenosti meritev in kasnejših izračunov.

7. ZAKLJUČEK

a) V študiji smo pokazali uporabnost DWI in DCEI kot kvantitativnih MR metod v neinvazivni oceni edema in prekrvljenosti v proksimalnem delu tibialnega tunela po rekonstrukciji ACL. V obeh skupinah bolnikov smo z DWI dokazali značilno upadanje ADC, z DCEI pa značilno upadanje gradienta in faktorja obarvanja v tibialnem tunelu en, dva in pol ter šest mesecev po rekonstrukciji, kar je v skladu s histološkimi najdbami manjšanja vnetnega odgovora v procesu vraščanja presadka.

b) Z DWI in DCEI, ki sta bili prvič uporabljeni v neinvazivnem sledenju vraščanja ACL v tibialnem tunelu pri človeku, smo dokazali značilno manjše vrednosti ADC en mesec po posegu ter značilno višje vrednosti gradienta obarvanja en in dva in pol meseca po rekonstrukciji ACL v tibialnem tunelu kot učinka lokalno apliciranega PRPG. Gre za neinvazivni MR dokaz znake manjšega edema oz. večje gostote celic ter večje gostote krvnih žil v tibialnem tunelu, kar je v skladu s histološkimi najdbami na živalskih modelih.

V prihodnosti pričakujemo nove modifikacije rekonstrukcije ACL. V tem kontekstu pričakujemo pomembno vlogo MRI in posebno DWI ter DCEI kot kvantitativnih neinvazivnih metod njihovega ocenjevanja.

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9. ZAHVALA

Poslanstvo diagnostičnega radiologa je odgovarjanje na klinična vprašanja s pomočjo svoje palete slikovnodiagnostičnih metod. Pričujoče delo je nastalo kot odgovor na vprašanje, kako bi neinvazivno ter hkrati kvantitativno ocenjevali učinek trombocitnega gela kot nove modifikacije artroskopske rekonstrukcije kolenske sprednje križne vezi na edem in prekrvljenost v tibialnem tunelu v okviru vnetnega odgovora v zgodnjem obdobju procesa vraščanja presadka.

Vprašanje je zastavil doc.dr. Matjaž Vogrin, takšnega odgovora, ki predstavlja prvo uporabo DWI in DCEI MRI v tem področju, pa ne bi bilo brez ideje mentorja prof. dr. Vladimirja Jevtiča. Profesor me je s pravšnjo kombinacijo temperamenta in hkrati umirjenosti, znanstvene in človeške širine, pa tudi humorja, usmerjal v celotnem poteku raziskave ter pri nastajanju člankov in disertacije. Somentor prof.dr. Igor Serša me je z natančnostjo in sistematičnostjo vodil pri načrtovanju preiskav in predvsem analiz dobljenih surovih podatkov MRI, kot tudi pri disertaciji in člankih. Brez radioloških inženirjev kot neposrednih izvajalcev MR preiskav le-teh ne bi bilo, še manj pa brez sodelovanja bolnikov, ki jim bodo, upam, rezultati te raziskave v prihodnosti koristili. Naj nazadnje, a ne najmanj, omenim tudi potrpljenje in odrekanje moje družine, ki ju je zahtevalo nastajanje doktorskega dela.

Vsem sem globoko hvaležen.

10. DELOVNI ŽIVLJENJEPIS

Rojen sem 4. aprila 1971, prihajam iz Maribora. Po končani osnovni in srednji šoli sem nadaljeval študij na medicinski fakulteti v Ljubljani. Po diplomi sem se l. 1999 zaposlil v Splošni bolnišnici Maribor. Po opravljenem sekundariatu sem opravil specializacijo iz radiologije na Kliničnem inštitutu za radiologijo Univerzitetnega kliničnega centra Ljubljana. Po specialističnem izpitu sem l. 2004 začel opravljati delo specialista radiologa na Oddelku za radiologijo Splošne bolnišnice Maribor, ki se je l. 2007 preoblikovala v Univerzitetni klinični center.

Ožji subspecialistični področji, s katerima se ukvarjam, sta muskuloskeletna ter intervencijska radiologija. Na področju muskuloskeletne radiologije se ukvarjam z rentgenskimi, računalniškotomografskimi, magnetnoresonančnimi in ultrazvočnimi preiskavami mišičnoskeletnega sistema, poleg tega pa opravljam tudi CT in MR artrografije rame, kolka, zapestja, komolca, kolena in gležnja. Opravljam tudi slikovno vodene citološke in histološke diagnostične punkcije ter biopsije.

Na področju intervencijske radiologije sem se poleg ostalega poglobil predvsem v zdravljenje kritične ishemije z rekanalizacijami dolgih okluzij povrhnje stegenske arterije in podkolenske perkutane transluminalne angioplastike. Pričel sem tudi z endovaskularnim zdravljenjem zapletov dializnih fistul.

Rezultate dela tako na področju muskuloskeletne kot tudi intervencijske radiologije spremljam ter predstavljam na strokovnih srečanjih. Redno obiskujem postspecialistične šole in tečaje s področja muskuloskeletne radiologije.

Sem glavni mentor za specializacijo iz radiologije ter neposredni mentor specializantom za področje muskuloskeletne radiologije.

Sem član Združenja radiologov Slovenije ter Evropskega združenja za radiologijo (ESR), Mednarodnega skeletnega združenja (ISS) ter član Komisije za šport Olimpijskega komiteja Slovenije.

Od leta 2006 sem asistent na katedri za radiologijo Medicinske fakultete v Mariboru ter sodelujem v pedagoškem procesu iz spoznavanja magnetne resonance, kardiovaskularne, intervencijske in muskuloskeletne radiologije.

11. IZJAVA DOKTORSKEGA KANDIDATA

UNIVERZA V MARIBORU

MEDICINSKA FAKULTETA

IZJAVA DOKTORSKEGA KANDIDATA

Podpisani Mitja Rupreht, vpisna številka 30803746,

izjavljam,

da je doktorska disertacija z naslovom **Uporaba dinamične magnetnoresonančne preiskave v sledenju vraščanja rekonstruirane kolenske sprednje križne vezi pri intraoperativni aplikaciji rastnih faktorjev**

□ rezultat lastnega raziskovalnega dela,

□ da predložena disertacija v celoti ali v delih ni bila predložena za pridobitev kakršnekoli izobrazbe po študijskem programu druge fakultete ali univerze, razen, kot opisano v točki 3

□ da so rezultati korektno navedeni in

□ da nisem kršil avtorskih pravic in intelektualne lastnine drugih.

Mitja Rupreht

Skeletal Radiol
DOI 10.1007/s00256-011-1256-6

SCIENTIFIC ARTICLE

Quantitative evaluation of the tibial tunnel after anterior cruciate ligament reconstruction using diffusion weighted and dynamic contrast enhanced MRI: a follow-up feasibility study

Mitja Rupreht · Vladimir Jevtič · Igor Serša · Matjaž Vogrin · Tomaž Šeruga · Marko Jevšek

Received: 13 March 2011 / Revised: 14 June 2011 / Accepted: 8 August 2011

Abstract
Objective The aim of the study was to evaluate the feasibility of two quantitative MRI methods: diffusion weighted imaging (DWI) and dynamic contrast enhanced imaging (DCEI), for follow-up assessment of the tibial tunnel after reconstruction of the anterior cruciate ligament (ACL).
Matherials and methods Twenty-three patients were examined by MRI at 1 and 6 months following ACL reconstruction. DWI and DCEI were utilized for evaluating the region of interest (ROI) within the proximal part of the tibial tunnel. From the resulting apparent diffusion coefficient (ADC) maps, ADC values were calculated. DCEI data were used to extract the enhancement factor (f_{enh}) and the enhancement gradient (g_{enh}) for the same ROI.
Results Calculated ADC as well as the f_{enh} and g_{enh} had diminished to a statistically significant extent by 6 months after ACL reconstruction. The average ADC value diminished from 1.48 (10^{-3} mm^2/s) at 1 month to 1.30 (10^{-3} mm^2/s) at 6 months after reconstruction. The average f_{enh} value decreased from 1.21 at 1 month to 0.50 at 6 months and the average g_{enh} value decreased from 2.01%/s to 1.15%/s at 6 months, respectively.
Conclusion The study proved feasibility of DWI and DCEI for quantitative assessment of the tibial tunnel at 1 and 6 months after ACL reconstruction. Both methods have the potential for use as an additional tool in the evaluation of new methods of ACL reconstruction. To our knowledge, this is the first time quantitative MRI has been used in the follow-up to the ACL graft healing process.

Keywords ACL graft · Healing · MRI · Diffusion weighted MRI · Dynamic contrast enhanced MRI

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Introduction

Rupture of the anterior cruciate ligament (ACL) is one of the most common knee injuries. After ACL reconstruction, stable graft-tunnel healing is desired, so that the graft tissue can be incorporated into the bone tunnel.

Two biological mechanisms take place after ACL reconstruction: healing in the bone tunnel and ligamentization of the intra-articular part of the graft. Graft healing in the tibial tunnel starts as an acute inflammatory response with edema, neutrophils, and recruited macrophages present in the tendon bone interface as early as 4 days after surgery. In the chronic phase of inflammation, monocytes and stem

Published online: 31 August 2011

cells initiate angiogenesis and the formation of a hypervascular granulation tissue interface between the graft and host bone [1]. Sharpey-like fibers, inserted directly into the bone, were found 12 weeks after the reconstruction in a dog model [2, 3]. During the ligamentization process, the graft at 6 weeks is completely covered by a vascular synovial envelope, and the intrinsic vasculature of the graft is complete at 20 weeks [4]. The tissue maturation process is finished at about 26 weeks [2, 5].

The maturation process was first assessed by MRI in 1991 [6]. Most of the MRI studies focused on the ligamentization process [6–10], graft length [11], imaging of the tendon harvest site [12], tibial tunnel width [11, 13], bone marrow edema encircling the tibial tunnel [9], or interference screw imaging [13]. A limited number of studies evaluated the intratunnel graft healing, using either qualitative [14] or semiquantitative [8, 10] assessment methods.

MRI proved to be an excellent noninvasive tool for the qualitative evaluation of edema and vascularity, which are both present during graft healing. Quantitative MRI sequences such as DWI and DCEI can provide additional quantitative information on these processes. DWI provides information on molecular motion of water, which enables calculation of the ADC. In the DCEI, fast imaging techniques, such as three-dimensional enhanced fast gradient echo (EFGRE3D) method, enable acquisition of multiple successive images of the same slice in short time frames and demonstrate signal intensity change after intravenous contrast agent administration. The value of both has been shown in other areas of musculoskeletal radiology [15–20]. To our knowledge, DCEI and DWI have not been used in the area of ACL reconstruction.

Therefore, the aim of this study was to assess the feasibility of the two quantitative MRI sequences, DWI and DCEI, for quantitative follow-up evaluation of postreconstructive tibial tunnel edema and vascularity.

Materials and methods

Patient selection and surgical technique

The study was designed as a 6-month, single center trial. The study was approved by the national ethics committee and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All patients gave their written informed consent for participation in the study.

Between February and June 2008, 25 patients (17 males, 8 females, mean age 33±12.5 years) were treated for ACL rupture. Patients with inflammatory diseases, diabetes mellitus, advanced knee osteoarthritis, severe meniscal injuries, previous knee surgery, allergy to the contrast agent, renal diseases, or thrombocytopenia were not included the study.

The procedures were performed by the same orthopedic surgeon. Following arthroscopic diagnosis of the ACL rupture, a routine arthroscopic reconstructive procedure was performed. In all cases, the single incision technique using a double-looped semitendinosus and gracilis tendon graft was used. The graft was inserted antegrade via the tibial and femoral tunnel and fixed with two bioabsorbable cross pins in the femoral tunnel and with one bioabsorbable interference screw in the tibial tunnel.

All patients followed the same rehabilitation protocol, with permission for immediate weight bearing and full range of motion.

Radiological assessment

The MRI scans were performed on a 1.5 T Signa Excite (General Electric, Waukesha, WI, USA) MRI scanner. Examinations were performed 1 and 6 months after ACL reconstruction.

DWI was performed with the echo-planar imaging (EPI) method, using the spin-echo single shot technique at TR/TE=8,000/75 ms. Two image acquisitions were

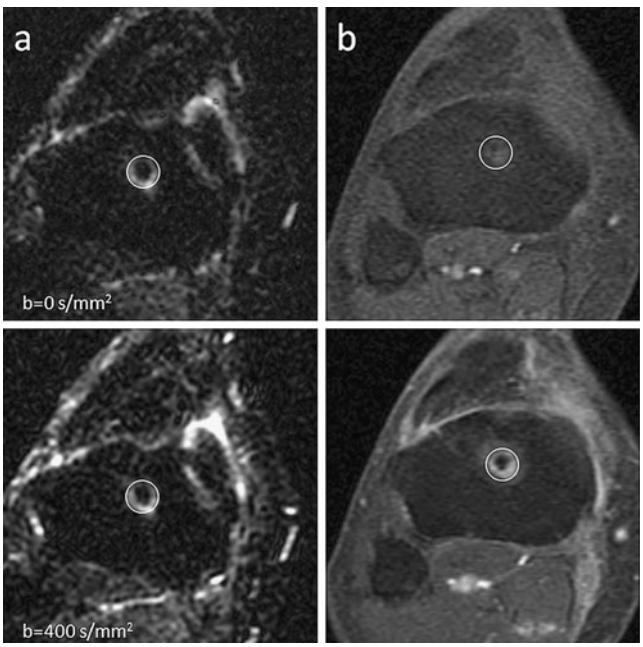

Fig. 1a, b Quantitative MR assessment of the tibial tunnel. Axial slices were oriented perpendicularly to the tibial tunnel. The slice half the distance between the plateau and the screw was chosen for the analysis. **a** Two oblique axial diffusion weighted images of the tibial tunnel performed with different diffusion strengths (b value). Regression analysis of both images allowed the calculation of the ADC for the selected ROI. **b** The first and last images from the stack of 25 successive dynamic contrast-enhanced images. Note the hyperintense interface zone in the encircled ROI in the last image

performed for each DWI method: one without (b=0 s/mm^2) and the other with diffusion weighting (b=400 s/mm^2). The area of the proximal tibia between the plateau and the interference screw was examined with 16 transverse slices perpendicular to the tibial tunnel (Fig. 1a). The tibial tunnel is easier to identify than the femoral tunnel on scout images, which facilitates planning in the paraaxial plane.

DCEI was performed by the EFGRE3D method. The paramagnetic contrast agent gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA) (Magnevist; Schering, Berlin, Germany) was administered intravenously using an MR injection system (Mississipi XD 2000, Ulrich, Germany) at a concentration of 0.1 mmol/kg body weight at a 3 ml/s flow rate, followed by an injection of 20 ml saline at the start of the first DCEI series. Images were acquired dynamically in 16 consecutive transverse slices through the proximal tibia perpendicular to the tibial tunnel (Fig. 1b) every 16 s in 25 time frames.

In postprocessing, an ROI within the tibial tunnel at a middistance between the plateau and the interference screw was examined. At this level, the images were free of artifacts because of the volume averaging of signals from the tibial plateau above and the screw below the ROI. Owing to the perpendicular orientation of slices to the tibial tunnel, the selected ROI was circular (Fig. 1a, b).

ADC maps were calculated from two DWI image sets of different b values, using the MRI Analysis Calculator plugin of the ImageJ image processing software (ImageJ, NIH, USA). This was followed by calculation of ADC values for the selected ROI.

DCEI images were also quantitatively analyzed with the ImageJ software. Each DCEI measurement yielded a time-intensity curve that was fitted using the OriginPro software (Origin Lab, Northampton, MA, USA) to a model function given by the equation:

$$SI(t) = \begin{cases} SI_0 \quad ; \quad t < \Delta \\ SI_0 + (SI_{\max} - SI_0)(1 - \exp(-(t - \Delta)/\tau)) \quad ; \quad t \geq \Delta. \end{cases}$$

The equation has four model parameters: SI_0 and SI_{max} correspond to the initial and plateau signals from ROI, while τ and Δ correspond to a characteristic time of contrast agent uptake and to a time delay between the start of DCEI imaging and the beginning of contrast agent uptake, respectively. Finally, two additional parameters were extracted from the best-fit parameters for each examination: the enhancement factor $f_{enh}=(SI_{max} - SI_0)/SI_0$, which is a dimensionless quantity and describes the amount of contrast accumulated in ROI compared to the baseline, and the enhancement gradient $g_{enh}=100[(SI_{max} - SI_0)/(SI_0\tau)]$, which yields a relative signal intensity increase per unit time, and therefore the enhancement rate.

Statistics

Numerical data are presented as mean values (standard deviation, SD), while categorical data are expressed as proportions. Measurements of the MRI parameters (ADC, f_{enh}, and g_{enh}) are expressed as estimated means and standard errors. Changes in the MRI parameters from the first to the final examination after the ACL reconstruction were analyzed by Wilcoxon's signed rank test. A *P* value less than 0.05 was considered statistically significant. Data were analyzed using the PASW 18 software (SPSS, Chicago, IL, USA).

Results

Following the arthroscopic procedure, patients were under routine clinical control and no complications were registered.

Two patients were lost from the follow-up; therefore 23 patients completed the initial and follow-up examinations.

ADC measurements

The average ADC value at 1 month after reconstruction was 1.48 (10^{-3} mm^2/s). At 6 months, the average ADC value was 1.30 (10^{-3} mm^2/s).

The statistically significant drop in the average ADC value in the cohort (−12%, $P<0.001$, Table 1) indicates decreased water proton mobility in the extracellular space at 6 months, compared to the first postoperative control.

DCEI measurements

In all examinations, the signal intensity within the selected ROI after the paramagnetic contrast administration first increased linearly, then reached a plateau and remained elevated until the end of signal acqusition, with no significant wash out from the area examined.

The average f_{enh} value decreased from 1.21 at 1 month to 0.50 at 6 months. The average g_{enh} value at 1 month was 2.01%/s and at 6 months, 1.15%/s.

Table 1 MRI parameters at follow-up examinations. Values are given as estimated mean values (standard error)

	Month 1	Month 6	Change	*P*
ADC (10^{-3} mm^2/s)	1.48 (0.03)	1.30 (0.02)	−12%	<0.001
f_{enh} (1)	1.21 (0.14)	0.50 (0.07)	−59%	<0.001
g_{enh} (%/s)	2.01 (0.24)	1.15 (0.16)	−43%	0.003

ADC Apparent diffusion coefficient value, f_{enh} enhancement factor, g_{enh} enhancement gradient

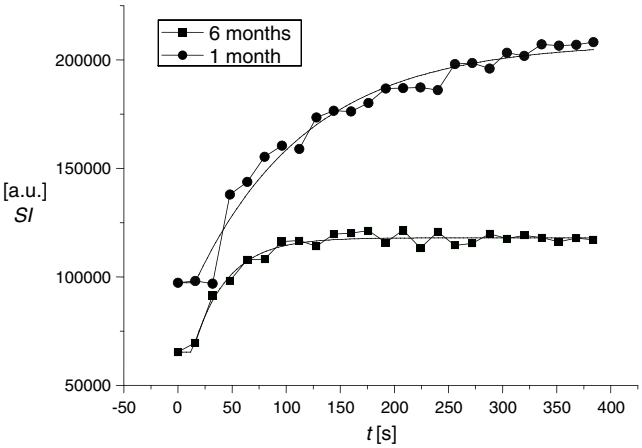

Fig. 2 A typical signal intensity time curve for the same patient at 1-month (*circles*) and 6-month (*squares*) controls, along with corresponding curves of the model function (*solid black line*). The experimental curves show an average signal intensity increase (y axis) in ROI after application of a contrast agent at 25 time intervals of 16 s (x axis). A diminished contrast uptake in the ROI at the second control is quite apparent

The decrease in f_{enh} (−59%; $P<0.001$) and g_{enh} (−42%; $P=0.003$) values was statistically significant (Table 1, Fig. 2). Both values reflect a decrease in the amount and rate of contrast uptake in the tibial tunnel during the healing process.

Discussion

Histological evaluation of ACL graft incorporation in humans is impossible for ethical reasons. Most animal studies focus more on the investigation of collagen, Sharpey-like fibers, or bone formation [1–3, 5]. There have been only a few histologic studies describing edema and vascularity [3, 4, 21]. However, angiogenesis is an important part of graft incorporation, particularly in the early phases [3, 4]. Research has focused on shortening the long rehabilitation period, and new operative techniques have been proposed to accelerate graft healing [10, 22, 23]. Moreover, it has been histologically proved that newer treatment methods provoke more extensive neovascularization compared with controls [22, 23]. Many qualitative and semiquantitative MRI methods have been proposed for the follow-up of ACL reconstruction. However, more precise evaluation requires the use of quantitative measurements. Therefore, the present study was designed to evaluate the feasibility of two quantitative MRI sequences for measuring postreconstructive tibial tunnel edema and vascularity in order to obtain additional information about these processes within the tibial tunnel.

In DWI, the image contrast is closely related to the random motion of water protons, which differs in various tissue environments. Diffusion weighting in the spin-echo echo-planar T2-weighted sequence is achieved by the use of two additional gradient pulses of equal magnitude and polarity symmetrically positioned relative to the refocusing RF pulse. The degree of diffusion weighting, i.e. the b value, is determined by the amplitude of the gradient pulses as well as by their duration and spacing. Two DWI acquisitions with different b values enable calculation of an ADC map. In areas of edema, higher ADC values correspond to elevated diffusion because of increased water content in the extracellular space where the motion of water is less restricted [24]. In our study, ADC values in ROI were higher in both controls than in the bone marrow (mean ADC=0.15×10^{-3} mm^2/s [25] to 0.23×10^{-3} mm^2/s [26]) and lower than that of free water (mean ADC=2.80×10^{-3} mm^2/s [27]). The drop in the ADC value 6 months after the procedure was significant, indicating restricted water proton motion in the extracellular space and also therefore the edema, compared to the first postoperative month, presumably due to the larger amount of fibrous granulation tissue produced during graft healing.

For qualitative assessment of vascularization in the interface zone as well as in the graft itself, intravenous contrast administration has been used since 1995 [7, 8, 28]. The possibility of acquisition of multiple successive images of the same slice in short time frames in DCEI permits quantitative measurements of the kinetics of paramagnetic contrast agent distribution in vivo in microvessels and in the interstitial space of the investigated tissues after a constant, pump controlled bolus injection [29, 30]. Previous studies have demonstrated its usefulness in the evaluation of bone marrow perfusion, showing correlations with microsphere blood flow measurements [31]. In the knee, the method has been described mostly in the assessment of synovitis [16], osteoarthritis [19], and tumors [20]. The temporal change of tissue signal following administration of the intravenous contrast agent reflects the complex process, related to the local blood supply and the extravasation of the contrast into the interstitial space [32]. In our study, the decrease in f_{enh} as a static enhancement parameter at the second control reflects the decrease in total extracellular space containing the intravascular volume and interstitial space. The decrease in the g_{enh} as a dynamic enhancement parameter at 6 months reflects the decline in the exchange rate between the intravascular and interstitial compartments, a rate which is influenced by microvessel permeability as well as by vascular density [16, 30]. Both parameters therefore correlate with decreased vascularity as part of the diminished inflammatory response in the process of graft incorporation, a correlation which has been histologically confirmed [1, 2, 4, 5]. There is no experience with the use of DCEI in the tibial tunnel, but according to Ostergaard,

although described in relation to synovitis, the g_{enh} in particular is even more closely related to inflammatory activity than the f_{enh} [16].

One of the limitations of our study relates to selection of ROI. The ROIs selected for our study encircled the tibial tunnel; therefore the ADC and enhancement values represent edema and vascularity in the interface zone as well as in the graft itself. According to the animal histological evidence, vascularization of the graft itself begins at 3–6 weeks after reconstruction. Therefore, in the first month, most of the DCEI values in ROI were probably related to the vascularized interface zone, whereas at 6 months, when graft vascularization should have been complete [2], these values were presumably in part also due to histological changes within the graft itself. Despite this, the graft generally remained visually hypointense on all MRI sequences. However, separate analysis of the interface zone would be more precise; on the other hand, because of the limited pixel number in smaller ROI, reproducibility of the measurements would be less accurate. Another limitation is the lack of histologic correlation with quantitative MRI data. The number of patients in our study is limited, mainly owing to the complexity of the measurements and data analysis. This therefore necessitates confirmation of the results by prospective studies on a larger number of patients.

To conclude, the results of our study prove the feasibility of both quantitative MRI methods, DWI and DCEI, in the evaluation of the complex process of ACL graft healing in the tibial tunnel at 1 and 6 months following reconstruction. Therefore both methods could be used as complementary quantitative noninvasive tools in the follow-up of ACL graft incorporation so we propose them as an additional quantitative MRI modality in future evaluations of new ACL reconstruction methods.

Conflict of interest The authors declare that they have no conflict of interest.

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Original Paper

European Surgical Research

Eur Surg Res 2010;45:77–85
DOI: 10.1159/000318597

Received: August 31, 2009
Accepted after revision: July 2, 2010
Published online: September 1, 2010

Effects of a Platelet Gel on Early Graft Revascularization after Anterior Cruciate Ligament Reconstruction: A Prospective, Randomized, Double-Blind, Clinical Trial

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Key Words
Anterior cruciate ligament reconstruction · Hamstring graft · Platelet-derived growth factors · Platelet gel · Revascularization

Abstract
Background: Slow graft healing in bone tunnels and a slow graft ligamentization process after anterior cruciate ligament (ACL) reconstruction are some of the reasons for prolonged rehabilitation. ***Aims:*** The purpose of this study was to determine if the use of platelet gel (PG) accelerates early graft revascularization after ACL reconstruction. ***Methods:*** PG was produced from autologous platelet-rich plasma and applied locally. We quantitatively evaluated the revascularization process in the osteoligamentous interface zone in the bone tunnels and in the intra-articular part of the graft by means of contrast-enhanced magnetic resonance imaging (MRI). ***Results:*** After 4–6 weeks, the PG-treated group demonstrated a significantly higher level of vascularization in the osteoligamentous interface (0.33 ± 0.09) than the control group (0.16 ± 0.09, $p < 0.001$). In the intra-articular part of the graft, we found no evidence of revascularization in either group. ***Conclusion:*** Locally applied PG enhanced early revascularization of the graft in the osteoligamentous interface zone after ACL reconstruction.

Introduction

Anterior cruciate ligament (ACL) rupture is one of the most common knee injuries. In case of symptomatic knee instability, operative reconstruction is the standard treatment, especially in the young, active population. The operative technique has been established >30 years ago [1]. There are several options concerning different autografts. A patellar tendon bone-tendon-bone graft was used in most of the cases in the 80s and 90s, but in the last 10 years the hamstring tendon graft has become popular and it has been used in >50% of all cases of ACL reconstruction worldwide [2–4]. A very important problem after operative reconstruction is the long rehabilitation period [5], especially in professional sportsmen. Before re-

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turning to full physical activity, a sufficient isokinetic state, especially quadriceps strength and proprioception [6], should be achieved, but improvement in graft healing in bone tunnels and the ligamentization process are crucial for facilitating an early and aggressive rehabilitation and ensuring rapid return to activity levels before injury [7, 8].

There are two desired biological processes after ACL reconstruction that take place – (a) graft healing in the bone tunnel and (b) ligamentizaton of the intra-articular part of the graft. Graft healing in the bone tunnel is a biological response after the surgical procedure. It starts with an acute inflammatory phase. The knee is immediately filled with blood from the drilled, exposed bone. In the bone tunnel, this generates an acute inflammatory response with mesenchymal cell recruitment, proliferation and matrix synthesis. The circulating platelets aggregate and degranulate within the forming fibrin clots, resulting in the release of cytokines [9]. In the chronic phase of inflammation, monocytes and stem cells initiate angiogenesis. The process is enhanced by high local concentrations of several platelet-derived growth factors (PDGFs) [10].

The pattern of change within the autograft tendon when transplanted into a human recipient has been described as graft ligamentization. It consists of necrosis, swelling, revascularization, fibroblastic invasion, synthesis and maturation of collagen fibers with ligament reformation [11].

The ACL autograft is strong immediately after surgery, weakens during the revascularization period between weeks 5 and 16, and then gradually regains its final strength after 6 months. The success of ACL reconstruction depends mostly on successful revascularization of the graft, which is required for the healing process in a bone tunnel and for the ligamentization process in the intra-articular part of the graft. Otherwise, the graft could not remain a viable substitute for ACL [12].

During the physiological cascades of soft tissue healing and bone growth, cellular and hormonal factors have a pivotal role [9], e.g. PDGFs, transforming growth factor-α/-β, epidermal growth factor, fibroblast growth factor 13, insulin-like growth factor, platelet-derived epidermal growth factor, platelet-derived angiogenesis factor, interleukin-8, tumor necrosis factor-α, connective tissue growth factor, granulocyte-macrophage colony-stimulating factor, keratinocyte growth factor and angiopoietin-2.

PDGFs have already been used for some indications in different fields of surgery, especially for wound and bone healing [13–17]. For the local application of PDGFs, platelet-rich plasma (PRP) is obtained by special devices from the autologous blood. The resulting PRP can be activated by thrombin to create platelet gel (PG). This solid gelatinous mass can be applied to the soft tissue, chronic wounds or bone [18, 19] in order to deliver PDGFs in loco and to accelerate physiological healing and tissue repair, especially angiogenesis [20].

The role of PDGFs after ACL reconstruction has been analyzed in previous studies in animal models, and results were evaluated with biomechanical tests and histological findings [21, 22]. In humans, results of early graft healing and revascularization were assessed by magnetic resonance imaging (MRI) [23–26]. There have been no studies investigating the factors that affect the speed of revascularization in ACL reconstruction.

Our study was designed to evaluate the effect of locally applied PG on the graft and bone tunnels during ACL reconstruction. We especially investigated early revascularization in the interface zone between the graft and bone tunnels and in the intra-articular part of the graft using quantitative contrast-enhanced MRI. A double-blind, randomized, prospective study on 50 patients was designed for this purpose.

We hypothesized that PG would enhance local vascularization in the interface zone in the tibial tunnel as well as in the intra-articular part of the graft reflected indirectly by a higher level of MRI enhancement.

Patients and Methods

Patient Selection and Randomization

The main indication for surgical reconstruction was a symptomatic unstable knee joint due to ACL rupture. The patients were between 18 and 50 years old. All patients with inflammatory diseases, diabetes mellitus, knee osteoarthrosis (3rd and 4th degree), severe meniscal injuries, previous knee surgery (osteotomies, ligament reconstruction, meniscal procedures and chondral lesion treatment), malignant diseases, allergy to the contrast media, renal diseases and thrombocytopenia were excluded from the study. The randomization by a computer-derived number generator (even number – PG, odd number – standard procedure) divided the patients into the PG group and the control group. In the PG group, PG was locally applied into the patients' tibial and femoral bone tunnels and onto the graft itself, whereas the patients in the control group did not receive any PG. From February 2008 to October 2008, 50 patients were treated, 25 (15 men and 10 women) in the PG study group and 25 (16 men and 9 women) in the control group. All patients were operated by the same operative technique and the same surgeon. All patients were blinded to the treatment. The results of the operations were monitored by an independent MRI expert in a blinded fashion. The study was approved by the National Ethics Committee of Slovenia.

Platelet Gel Preparation

The Magellan (Medtronic Biologic Therapeutics and Diagnostics, Minneapolis, Minn., USA) autologous platelet separator was used to prepare PG according to the manufacturer's instructions. This closed system is assumed to reduce operator error and decrease potential contamination. After the arthroscopically established ACL rupture, 52 ml of the patient's whole blood was drawn into an especially designed disposable separation chamber and mixed with 8 ml of 10% calcium citrate. The mixture was then placed inside the closed system. The final volume of autologous PRP was set to 6 ml. Two custom-designed syringes were placed into the Magellan system adjacent to the centrifuge separator. The centrifuge separator and the syringes were connected to a sterile tubing system and the autologous PRP was delivered through an automatic pump, which ensured accurate delivery with minimum platelet activation. The resultant 6 ml of autologous PRP were then mixed with activated autologous human thrombin (fig. 1) and applied to the area of interest (fig. 2), where the platelets were activated and an autologous PG was formed in 2 min, as reported earlier [21, 22].

Surgical Technique

The same surgeon (M.V.) performed all procedures. Routine arthroscopic revision and diagnosis, i.e. ACL rupture, was followed by a reconstructive procedure. In all cases, we used the single-incision technique with the double-looped semitendinosus and gracilis tendon graft. The drill tunnels in the tibia and femur were 7–9 mm in diameter. The tunnel size was matched to the cross-sectional size of the graft. The graft was inserted anterograde via the tibial and femoral tunnel and fixed with 2 bioabsorbable cross pins (Mitek, 3.2 mm) in the femoral tunnel and with 1 bioabsorbable interference screw (Mitek, 8–10 mm) in the tibial tunnel (fig. 2). After autograft positioning, PG was applied into the femoral and tibial tunnels as well as onto the graft itself.

Platelet Gel Application

The double-looped ST-G tendon graft was covered with 4 ml of freshly mixed autologous PRP with the autologous human thrombin that was followed by clot formation. Then the graft was pulled into the bone tunnels and fixed on the femoral side with the cross pins. After fixation on the femoral side, 1 ml of PG was injected into the femoral tunnel and tibial tunnel, respectively, between the strands of the graft (fig. 2). Since the infiltration areas in both tunnels were relatively small ($V = l\pi r^2$, where V = volume, r = radius and l = tunnel length; V = 1.15 ml on tibial and femoral sides) and already filled with soft tissue graft, pressure was applied to force PG into the graft and also into the surrounding cancellous bone. PG remnants were applied onto the intra-articular part of the graft, which was already covered with gel before positioning in the tunnels. At that point of the operation, we disconnected water inflow to additionally prevent PG washout. An interference screw was inserted after the PG application. The procedure was performed under arthroscopic control using a slight modification of the previously published PG application protocol [27].

Contrast-Enhanced MRI Assessment

Contrast-enhanced MRI studies were carried out 4–6 (K1) and 10–12 weeks (K2) after surgery. MRIs were made in T_1 sequence

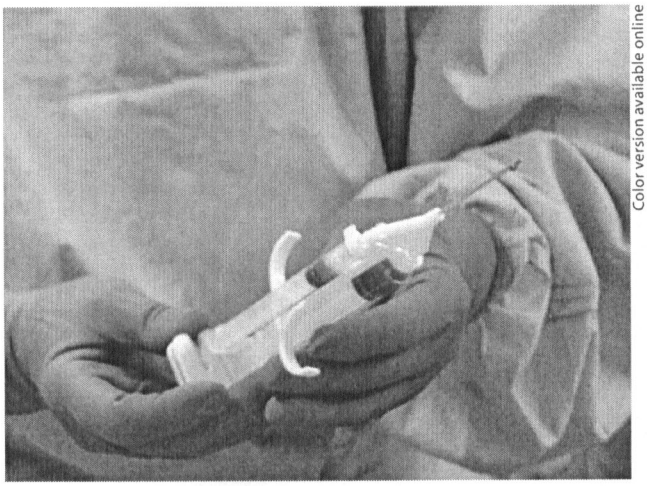

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Fig. 1. Autologous PRP and activated human thrombin before application.

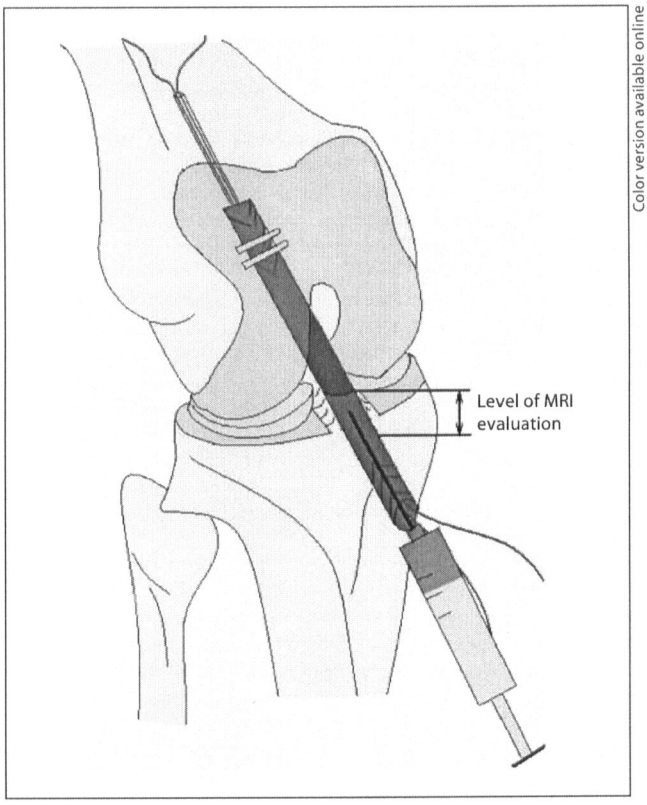

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Fig. 2. PG application after autograft positioning. PG was applied to the osteoligamentous interface in the tibial and femoral bone tunnels (blue color; color refers to online version only). Before positioning, the graft was covered with autologous PRP in combination with autologous human thrombin until the clot was formed.

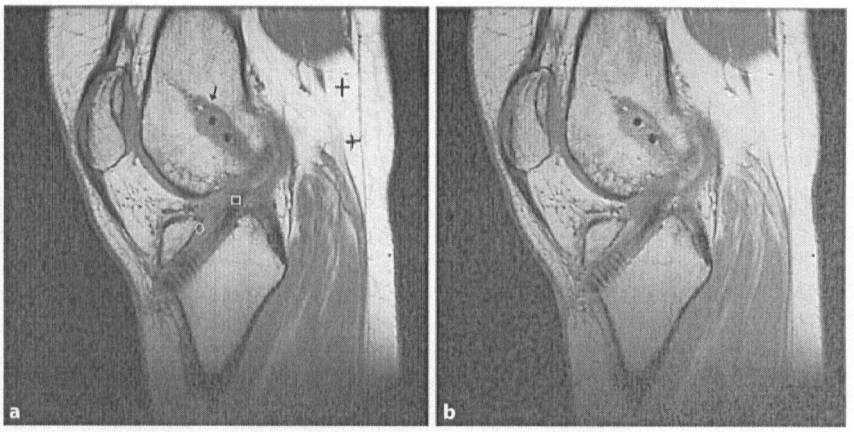

Fig. 3. MRI evaluation of vascularization. The oblique sagittal image in the T_1 sequence before (**a**) and after (**b**) application of the paramagnetic contrast medium showing positions of the osteoligamentous interface (O), intra-articular part (□) of the graft and background (+) ROIs for signal intensity measurements. Note the enhancement in the osteoligamentous interface zone (O) and in the interface in the femoral tunnel (↓).

(TR 675, TE 18, slice thickness 2.5, spacing 0.3 mm, 3 NEX, matrix 320 × 224) in the modified sagittal plane before and 10 min after the intravenous administration of the paramagnetic contrast medium Gd-DTPA (Magnevist; Shering, Berlin, Germany) at a dose of 0.2 mmol/kg body weight.

The vascularization rate was defined as the MRI signal enhancement of the osteoligamentous interface between the wall of the tibial tunnel and the graft and in the intra-articular part of the graft itself after paramagnetic contrast medium administration. Circular 0.1-cm^2-large regions of interest (ROI) were placed in the interface zone and in the intra-articular part of the graft itself. We decided to evaluate the level of vascularization only at the tibial side due to technical problems with MRI investigation at the femoral side. The reason lies in the fixation method – cross pins on the femoral side close to the joint line prevented good MRI evaluation without artifacts, especially for refined quantitative revascularization measurements. Circumstances were opposite at the tibial site, with an interferential screw 2.5 cm away from joint line. The other reason was in fact that tibial fixation failure occurs more commonly at the tibial tunnel [28]. This low ultimate load may be influenced by low bone density of the tibia in comparison with the femur at tunnel level. Therefore, we found the tibial side more important to evaluate biological processes than the femoral side.

Because of different physiological conditions such as hydration, the background signal was also measured. Background measurements were calculated from the mean value of two measurements of an identical ROI placed in the posterior subcutaneous fat. The result of one measurement was expressed as a signal/noise quotient (SNQ). The vascularization rate was defined as the difference in the SNQ after and before the application of the contrast medium for each ROI, as described by Howell et al. [24] and Vogl et al. [29]. In other words, the more vascularized the ROI is, the greater the difference in the MRI signal intensity and therefore the greater the difference between the SNQ after and before the application of the contrast medium. In the ROI with no difference in the SNQ before and after application of contrast medium, we can presume that there is no vascularization. We also measured the diameters of the tibial and femoral tunnels 1 cm from the joint line at the first and second control follow-ups. All MRI images were evaluated by a musculoskeletal radiologist who was blinded to treatment.

Other investigation methods, such as clinical examination, functional scoring (Tegner-Lysholm, International Knee Documentation Committee), radiographs and knee stability testing by KT-2000 may also be of great help in the evaluation of late PG effects but not crucial in early revascularization assessment. In our opinion, contrast-enhanced MRI was the only option available for quantitative evaluation of this biological process.

Rehabilitation

All patients followed the same rehabilitation protocol, with the permission of immediate weight bearing and full range of motion [19]. No rehabilitation brace was used postoperatively. The importance of reaching full extension was emphasized from the beginning. Closed kinetic chain exercises were started immediately after the surgical procedure. Running was allowed at 12 weeks and contact sports at 6 months provided that the patient had no knee joint effusion, achieved a full range of motion and obtained muscle strength of at least 90% compared to the contralateral leg.

Statistical Methods

Background data statistics included percentage distributions, mean values and standard deviations. Differences in characteristics between the two therapeutic methods were compared by χ^2 tests for categorical variables and by the Mann-Whitney rank tests for continuous variables. Linear regression was used to adjust the effect of age and body mass index (BMI) on the two therapeutic methods.

Results

Fifty-eight patients were assessed for eligibility, but 8 of them were excluded from the study because of knee osteoarthrosis. Fifty patients were allocated to one of the two groups after randomization. Twenty-five were included into the PG group and 25 into the control group.

Twenty patients (15 men and 5 women) of the 25 included into the control group, and 21 (13 men and 8 women) of the 25 included into the PG group were available for the follow-up and were analyzed. Altogether 9 of 50 patients were lost to follow-up. Among them, 3 patients (1 because of another injury on the same knee and 2 because of disagreement to participate in the study any longer, without adequate explanation) in the PG and 3 patients (2 because of medical conditions which were not related with the knee surgery and 1 because of disagreement to participate in the study any longer, without explanation) in the control group failed to respond to the MRI evaluation at the proper time. In 2 patients in the control group we did not finish the MRI evaluations due to technical problems (an error occurred during the intravenous administration of the paramagnetic contrast medium), and 1 patient in the PG group had an allergic reaction. A comparison of the preoperative parameters between the PG and control groups showed that both groups were comparable in gender, age, injury site, BMI and preoperative platelet counts (table 1).

Platelet counts were also determined in the individual PRPs, which were used for PG formation. The average platelet count in the PRP was 978 × 10^9/l (ranging from 552 to 1,326 × 10^9/l). The average blood platelet count was 190 × 10^9/l in the PG group (ranging from 152 to 240 × 10^9/l) and 207 × 10^9/l (ranging from 161 to 249 × 10^9/l) in the control group.

For platelets counts, whole blood was sampled 1 day before surgery and PRP just after the process in a platelet separator and counted using an electronic hematological counter (Celly 70; Biocode-Hycel, Rennes, France). Before counting in the electronic counter, samples were investigated microscopically to avoid potential errors due to platelet aggregation.

We measured the intensity of the MRI signal in the osteoligamentous interface, intra-articular part of the graft and in the background. Measurements were performed in all patients of both groups before and after application of the contrast medium at the first and second follow-ups (K1, K2; table 2).

We quantitatively evaluated differences in early vascularization between the PG and the control groups, both in the interface zone in the bone tunnels as well as in the intra-articular part of the graft (table 3; fig. 4). The calculated vascularization rate in the tibial tunnel at the first control at 10–12 weeks (V_{tib}K1) was 0.16 ± 0.09 in the control group and 0.33 ± 0.09 in the PG group. Based on the significant difference between the two methods ($p < 0.001$), we can conclude that PG has significantly enhanced early graft revascularization in the osteoligamentous interface. At the second control, the calculated vascularization rate (V_{tib}K2) was 0.17 ± 0.10 in the control group and 0.20 ± 0.13 in the platelet group. The dynamics of vascularization in both groups is shown in figure 4.

Table 1. Preoperative data of knee-injured patients

Characteristics	Control group (n = 20)	PG group (n = 21)	p value
Sex			0.505
Male	15	13	
Female	5	8	
Age, years	32.6 ± 12.3	37.2 ± 8.4	0.112
Injured knee, %			1.000
Right	60.0	57.1	
Left	40.0	42.9	
BMI	24.5 ± 2.1	26.5 ± 4.1	0.078

Table 2. MRI signal intensity (S) in the PG group and the control group before and after the application of the contrast-enhanced medium (CM)

Groups	S_{tib} K1	S_{art} K1	S_{noise} K1	S_{tib} K2	S_{art} K2	S_{noise} K2
PG group						
Before CM	290.9	94.8	1,029.8	283.9	108.2	1,098.2
After CM	657.7	91.9	1,074.6	527.7	132.1	1,198.9
Control group						
Before CM	311.3	66.7	1,141.1	279.4	79.4	1,167.8
After CM	509.6	77.8	1,177.8	494.6	102.5	1,201.9

K1 = First control (4–6 weeks); K2 = second control (10–12 weeks); tib = tibial osteoligamentous interface; art = intra-articular part of the graft; noise = fat tissue background.

Table 3. Vascularization rate in the interface zone between the graft and bone tunnels (V_{tib}) and in the intra-articular part of the graft (V_{art}) at the first and second control follow-ups

Vascularization rate	Control (n = 20)	PG (n = 21)	p value
V_{tib}K1 (4–6 weeks)	0.16 ± 0.09	0.33 ± 0.09	<0.001
V_{tib}K2 (10–12 weeks)	0.17 ± 0.10	0.20 ± 0.13	0.404
V_{art}K1 (4–6 weeks)	0.01 ± 0.02	−0.01 ± 0.08	0.262
V_{art}K2 (10–12 weeks)	0.02 ± 0.04	0.01 ± 0.06	0.404

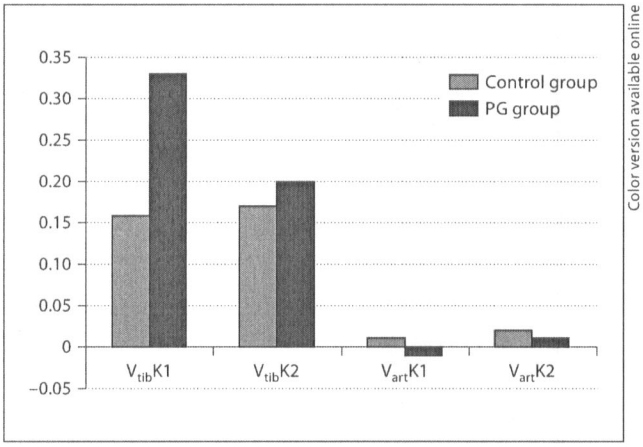

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Fig. 4. Dynamics of the vascular state in the osteoligamentous interface and intra-articular part of the graft. Note the high level of vascularization at the osteoligamentous interface (V_{tib}) in the PG group at the first control and decreased vascularization at the second control, contrary to the dynamics in the control group. There is no sign of vascularization in the intra-articular part of the graft (V_{art}) at the first control, and just a slight improvement at the second control.

Table 4. Cross-sectional diameters (TD) of tibial and femoral tunnels at first and second control follow-ups

Tunnel	Cross-sectional diameter, cm^2		
	Control group (n = 20)	PG group (n = 21)	p value
Tibial			
TDK1 (4–6 weeks)	0.96 ± 0.32	0.86 ± 0.33	0.34
TDK2 (9–11 weeks)	0.93 ± 0.22	0.86 ± 0.34	0.44
Femoral			
TDK1 (4–6 weeks)	0.77 ± 0.20	0.71 ± 0.15	0.28
TDK2 (9–11 weeks)	0.90 ± 0.27	0.83 ± 0.19	0.32

Table 5. Linear model to calculate $K1_{tib}$ showing the independence of the results concerning BMI and age

	β	p value	95% confidence interval
Therapy method	−0.698	<0.001	−0.237, −0.111
Age	0.105	0.420	−0.002, 0.004
BMI	−0.130	0.330	−0.015, 0.005

It is obvious that there is a decrease in vascularization in the PG group at the second control, representing the replacement of vascular tissue with hypovascular scar tissue in the interface zone between the graft and the bone tunnels.

The calculated vascularization rate in the intra-articular part of the graft at the first control demonstrated no vascularization at all in both groups ($V_{art}K1$: 0.01 ± 0.02 and −0.01 ± 0.08, respectively). The results of the second control ($V_{art}K2$: 0.02 ± 0.04 and 0.01 ± 0.06, respectively) suggested initiation of revascularization, but without a statistical significance between both groups.

At the femoral and tibial sides, we found initial enlargement in the control group in comparison with the PG group at the first and also at the second control follow-ups. The difference between the groups was not statistically significant (table 4).

In order to make sure that the patients' age and BMI did not have any significant effect on the results, we used linear regression to calculate the $V_{tib}K1$ value (dependent variable) in relation to the therapeutic method (control group vs. experimental group) and BMI. The results have shown that the significant variable was the therapeutic method, while age and BMI were not in any correlation with $V_{tib}K1$ (table 5). The multiple correlation coefficient (R) has shown a strong overall linear association between the independent variables and $K1_{tib}$ ($R = 0.695$; $p < 0.001$).

Discussion

To our knowledge, studies investigating the effect of PG on graft revascularization after ACL reconstruction in humans have not been published to date.

The process of graft revascularization has been described in detail only in animal models. In a study by Kohno et al. [30], the clinical outcome of ACL reconstruction using a hamstring tendon graft depended on the biological integration between the tendon and the bone. The healing process starts immediately after the operation. The empty space between the tendon and the bone is replaced with an increasing number of fibroblasts in a few days. At 3 weeks, small vessels appear along with an increased number of osteoblasts on the bone surface. After 6 weeks, the vessels decrease in number along with a shield-like new bone formation surrounding the tendon and with an increased number of collagen fibers integrated along the tendon. At 12 weeks, the tendon is practically directly attached to the bone.

Due to technical difficulties, knowledge about the ligament graft healing process in humans is limited, but available data have shown that complete integration of the graft into the surrounding bone occurs as early as 12 weeks postoperatively.

Arnoczky et al. [31] investigated revascularization of patellar tendon grafts after ACL reconstruction in dogs. Their results showed that the intra-articular part of the graft was initially avascular, but it was surrounded by synovial tissue with abundant blood vessels after 6 weeks. Revascularization originated from the soft tissue of the infrapatellar fat pad and the posterior soft tissue of the joint. The graft had the vascularity and collagen pattern of native ACL 1 year after surgery. Unterhauser et al. [32] investigated revascularization of the flexor tendon graft after ACL reconstruction in sheep. Their study showed that vascularization was found after 6 weeks, reaching the vascular status of native ACL after 24 weeks. Kuroda et al. [33] studied the role of PDGFs in the regulation of the graft healing process. They stimulated angiogenesis, cell proliferation and collagen synthesis. Increased levels of PDGFs were observed within the healing area in the early postoperative period, peaking 3 weeks after implantation. Thereafter, the concentration of PDGFs decreased and returned to the preoperative level 12 weeks postoperatively. Our study was designed based on this knowledge and encouraged with animal model studies [21, 22], which demonstrated positive effects of PG in biological processes after ACL reconstruction.

In the literature, we found several trials assessing graft healing after ACL reconstruction using MRI evaluation in humans [29, 33]. Few studies correlated the signal intensity of the graft to MRI and corresponding histological findings during early graft healing. Weiler et al. [35] found that the graft was fully revascularized after 3 months and that the better the revascularization process, the stronger the graft became. Orrego et al. [27] assessed the role of PG in graft integration on MRI scans 3 and 6 months postoperatively. They concluded that the use of PG had an enhancing effect on the graft maturation process evaluated only by MRI signal intensity, without showing any significant effect in the osteoligamentous interface or tunnel widening.

Based on the importance of the revascularization process after ACL reconstruction, we decided to evaluate the role of PG on ACL graft revascularization in the first 12 postoperative weeks. We think that graft integration is a complex and gradual process and not a cascade of events. Therefore, we evaluated graft revascularization using contrast-enhanced MRI, which is both a qualitative and quantitative method.

Our first prediction was that the level of vascularization in the interface zone in the PG group should be higher than in the control group, as monitored at the first MRI control 4–6 weeks postoperatively. Results have confirmed our predictions at that point. At the second follow-up (10–12 weeks) we expected a decreased MRI signal in the tibial interface zone in both groups, since at 3 months the vascular tissue should be already replaced by low-vascular collagenous scar tissue. Indeed that happened in the PG group but not in the control group, in which the level of vascularization was higher than 4–6 weeks after surgery. Those data indicate that the process of graft healing after ACL reconstruction is slower than previously estimated and that the process of revascularization in the tibial interface zone does not reach its peak at 3 months, but later.

Our second prediction was that revascularization of the intra-articular part of the graft should be more pronounced in the PG group than in the control group. This was not confirmed, furthermore our data suggest that revascularization in the intra-articular part of the graft did not at all start 4–6 weeks after surgery, and it was only minimal 10–12 weeks after surgery. No statistically significant differences were found between the two groups. Those data indicate that the ligamentization process starts much later than the graft healing process in the bone tunnel itself, and that the PG applied locally during the operative procedure does not have an enhancing role at that point.

Limitations

We are aware of certain weaknesses of our study. For a better statistic evaluation, a larger number of patients should have been evaluated. Secondly, MRI evaluation alone can neither document the healing in the bone tunnel nor the process of revascularization with the same precision as, for instance, histological examination. We could only indirectly correlate our results with potential histological changes. Histological specimens of the graft in correlation with the MRI results would be preferable, but in human studies that would be too difficult to justify. It would also be better to repeat contrast-enhanced MRI more often during the study in order to determine the healing process more precisely. A major problem for future studies represents PG application in the intra-articular part of the graft. Washout of the platelet gel from that area is a great risk, much higher than in bone tunnels. We are still looking for technical solutions to solve

this problem. There are some potential solutions, such as gene therapy, repetitive intra-articular PG injections, use of a biodegradable drug delivery tool or vascular grafting, but scientific confirmation is lacking to date. Another problem represents the dosage of the PG applied to the graft tissue and into the bone tunnels. There are no studies available concerning dosage and timing of PG application for this specific indication, as for example in medial collateral injury healing [35]. Finally, it would be ideal to observe the revascularization of the intra-articular part of the graft for a much longer period.

Conclusion

The PG used for ACL reconstruction enhances early revascularization in the interface zone between the graft and the bone tibial tunnel. This could shorten the period of rehabilitation after surgery, but before final conclusions can be drawn, further studies are required, especially concerning the dosage and time of PG application, 'washout' problems in the intra-articular part of the graft, the integrity of revascularization, maturation of the intra-articular part of the graft and the formation of sclerotic bone around the tunnel, as well as comparisons of those data with clinical findings and knee stability.

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Wien Klin Wochenschr (2010) 122 [Suppl 2]: 91–95
DOI 10.1007/s00508-010-1340-2

Printed in Austria

Wiener klinische Wochenschrift
The Middle European Journal of Medicine

The effect of platelet-derived growth factors on knee stability after anterior cruciate ligament reconstruction: a prospective randomized clinical study*

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Summary. *Background:* Arthroscopic reconstruction is a standard surgical procedure in cases of symptomatic knee instability due to rupture of the anterior cruciate ligament. Bone-tendon-bone and hamstring tendon grafts are both in use for anterior cruciate ligament reconstruction. There are no significant differences between the two types of graft in relation to function scores, but there is a difference in anteroposterior stability when measured on the KT-2000 arthrometer: knee joints after reconstruction with bone-tendon-bone autografts are more stable than those reconstructed with hamstring tendon autografts.

Purpose: To improve knee stability after anterior cruciate ligament reconstruction with a hamstring graft and use of platelet-derived growth factors.

Basic procedure: Platelet-leukocyte gel was produced from platelet-leukocyte-rich plasma prepared from a unit of whole blood in an autologous platelet separator. The gel was applied locally, after hamstring graft placement. Fifty patients were included in the study: 25 in the platelet gel group, 25 in a control group. We evaluated anteroposterior knee stability with the KT-2000 arthrometer before surgery and at 3 and 6 months after surgery.

Main findings: Patients treated with the gel demonstrated significantly better anteroposterior knee stability than patients in the control group. The calculated improvements in knee stability at 6 months were 1.3 ± 1.8 mm in the control group and 3.1 ± 2.5 mm in the platelet gel group ($P = 0.011$).

Principal conclusion: Platelet-leukocyte gel, applied locally, can improve knee stability in surgery for reconstruction of the anterior cruciate ligament.

Key words: Platelet-gel, knee stability, anterior cruciate ligament.

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Introduction

Rupture of the anterior cruciate ligament (ACL) is one of the most common knee injuries. In cases of symptomatic knee instability, surgical reconstruction is the standard treatment, especially in a young active population. The operative technique is well known and has been established for more than 30 years [1]. There are several options concerning different autografts but the patellar bone-tendon-bone (BTB) graft still represents the gold standard, and in the past 10 years the hamstring tendon (HT) graft has become increasingly popular and is used in more than 50% of all ACL reconstruction cases worldwide. Many clinical analyses have compared these two techniques [2, 3].

The disadvantages of patellar autografts are the risk of damage to the extensor apparatus, potential increase of patellofemoral pain and retained weakness of the patellar tendon. The disadvantages of HT graft are potential weakness of the hamstring muscle and slower healing of the graft attachment site. There are no significant differences between BTB and HT grafts with regard to function scores (Tagner, IKDC, Lysholm) after 6 and 12 months, but there is a difference in anteroposterior stability when measured on the KT-2000 arthrometer (MEDmetric, San Diego, CA, USA): knee joints after reconstruction with BTB autografts are more stable than those reconstructed with HT autografts [4].

Two important biological mechanisms are in process after ACL reconstruction with an HT graft: healing and ligamentizaton. Graft healing in the bone tunnel is a biological response after the surgical procedure. It begins upon release of the tourniquet when the knee is immediately filled with blood from the drilled exposed bone. This generates an acute inflammatory response with mesenchymal-cell recruitment, proliferation and matrix synthesis. There is aggregation and degranulation of circulating platelets within the forming fibrin clot, resulting in controlled release of cytokines. In particular, transforming growth-factor beta and platelet-derived growth factors (PDGFs) act in a coordinated manner to regulate this early response. Neutrophil recruitment peaks at

about 24–48 hours after operation and is followed by increasing representation of monocytes, which are essential for clot maturation, tissue adherence to bone and early formation of granulation tissue [5]. In the chronic phase the monocytes drive angiogenesis. The process is enhanced by high local concentrations of some thrombocyte-derived growth factors and hypoxia by proliferating and synthesizing new extracellular matrix to replace the injured tissue with connective scar tissue. Matrix synthesis may last for several weeks. The fibrotic process is represented by recruiting fibroblasts and stimulating their synthesis of collagens I, III and V, proteoglycans and fibronectin. The remodelling phase, when collagen is synthesized, degraded and reorganized as it is stabilized by molecular crosslinking into a scar, is also cytokine mediated. The regulation of enzymes and their inhibitors ensures tight control of local proteolytic activity [6].

The pattern of change within the body of an autograft tendon when it is transplanted into a human recipient has been described as ligamentization, a process of necrosis, swelling, revascularization, fibroblastic invasion and synthesis and maturation of collagen fibers with ligament reformation. Nevertheless, biomechanical studies in animal models have shown that the strength and stiffness of the grafted tissue never reaches that of native ACL [7].

During the physiological cascades of soft tissue healing and bone growth, cellular and hormonal factors have pivotal roles, particularly PDGFs [8], some of which are described in Table 1.

PDGFs have already been used for some indications in several fields of surgery, particularly in wound healing and bone healing [9–13]. There are also studies analyzing the role of PDGFs after ACL reconstruction, using animal models [14].

Intraoperatively, specialized devices fractionate autologous blood into red blood cells, platelet-poor plasma and platelet leukocyte-rich plasma; the latter can then be activated with autologous thrombin to create a viscous solution termed platelet-leukocyte gel (PLG). The gel can be applied exogenously as a solid gelatinous mass to soft tissues, chronic wounds, bone, When PLG is applied to tissue, PDGFs are delivered, mimicking and accelerating physiological healing and reparative tissue processes [15].

We designed our study to determine the potential effect of locally applied PDGFs on knee stability after ACL reconstruction.

Table 1. Some of the most important platelet-derived growth factors in reconstruction of the anterior cruciate ligament*

Platelet growth factor	Function
Transforming growth factor-β (TGF-β)	Stimulates mesenchymal cell proliferation; regulates endothelial, fibroblastic and osteoblastic activity; stimulates endothelial chemotaxis and angiogenesis
Basic fibroblast growth factor (bFGF)	Promotes growth and differentiation of chondrocytes and osteoblasts
Platelet-derived growth factor (PDGF)	Mitogenetic for mesenchymal cells and osteoblasts; regulates collagenase secretion and collagen synthesis
Epidermal growth factor (EGF)	Stimulates endothelial chemotaxis, angiogenesis, regulates collagenase secretion
Vascular endothelial growth factor (VEGF)	Increases angiogenesis and vessel permeability
Connective tissue growth factor (CTGF)	Promotes angiogenesis, cartilage regeneration, fibrosis and platelet adhesion

*Knee Surg Sports Traumatol Arthrosc (1999) 7: 9–14.

Patients, materials and methods

Patient selection

The main indication for surgical reconstruction was a symptomatic unstable knee joint resulting from ACL rupture. All patients were between 18 and 50 years old. We excluded patients with inflammatory diseases, diabetes mellitus, developed knee osteoarthrosis, malignant diseases, allergy to contrast media, renal diseases and thrombocytopenia. Patients were allocated numbers and randomized into two groups: those with an even number received PLG; odd numbers received the standard procedure. Between February and June 2008, 50 patients were operated on, 25 in each group. All patients were operated on by the same surgeon (MV) using the same operative technique, the only difference being that patients in the PLG group received the gel locally in the tibial and femoral bone tunnels and into the graft itself, whereas patients in the control group did not receive any PLG. All patients gave their written informed consent to participate. The study was approved by the National Ethics Committee.

Preparation of the platelet-leukocyte gel

The Magellan autologous platelet separator (Medtronic Biologic Therapeutics and Diagnostics, Minneapolis, MN, USA) was used for PLG preparation (Fig. 1). This closed system is purported to reduce operator error and decrease potential contamination. The process begins, after an arthroscopically established ACL rupture, when 52 ml of the patient's whole blood is drawn and loaded into a specially designed disposable separation chamber and mixed with 8 ml 10% calcium citrate acting as anticoagulant. The mixture is then placed inside the closed system. The operator can

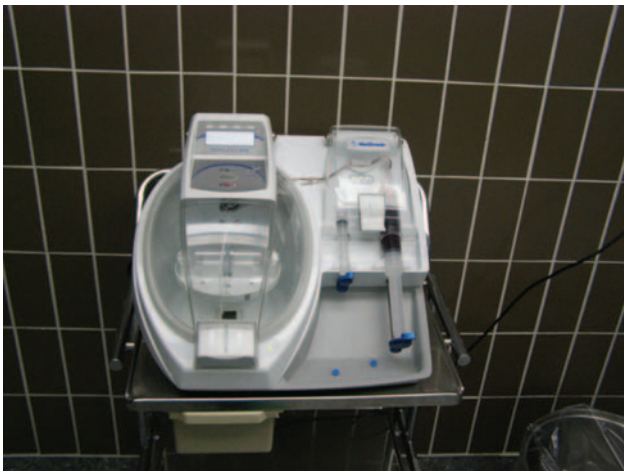

Fig. 1. Magellan autologous platelet separator

determine the volume of autologous platelet- and leukocyte-rich plasma. In our study that was 6 ml. Two custom-designed syringes are placed in the Magellan system adjacent to the centrifuge separator. Sterile tubing connects the centrifuge separator and the syringes through a closed system and delivers the autologous platelet-rich plasma through an automatic pump. This ensures accurate delivery with minimum platelet activation. The platelet-rich plasma is then combined with activated human thrombin and applied to the area of interest, where platelets are activated and autologous PLG is created [17, 18].

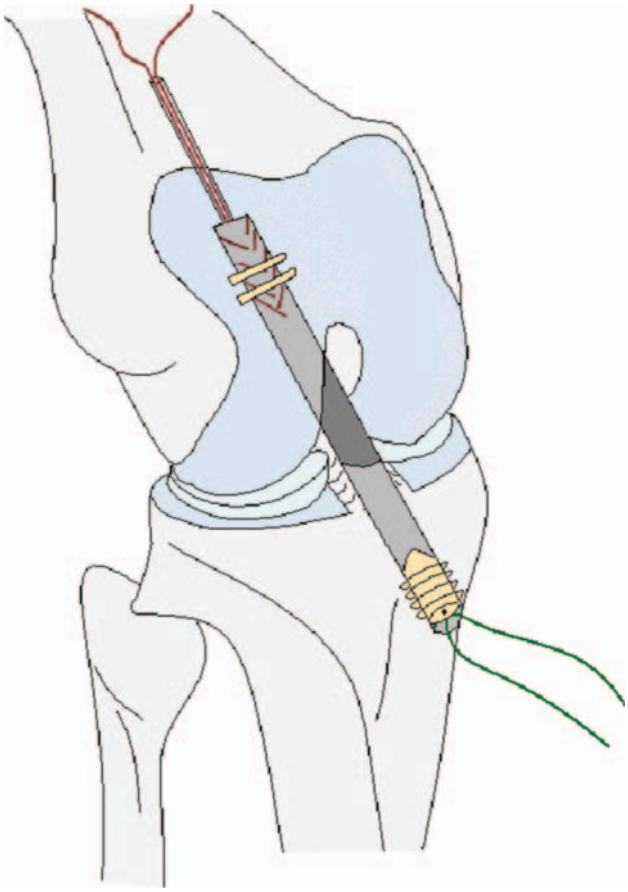

Fig. 2. Position and fixation of the autograft in the femoral and tibial tunnels

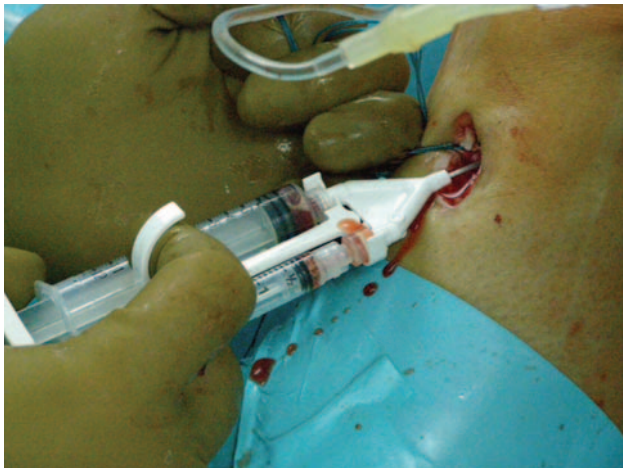

Fig. 3. Application of platelet-rich plasma and human thrombin

Surgical technique

All procedures were performed by the same surgeon (MV). After routine arthroscopic revision and establishment of the diagnosis (ACL rupture), the reconstructive procedure followed. In all cases a single incision technique with double-looped semitendinosus and gracilis tendons was used. The drill tunnels in the tibia and femur were 7–9 mm in diameter and matched to the cross-section size of the graft. The graft was inserted anterograde via the tibial and femoral tunnels and fixed with two bioabsorbable cross pins (Mitek 3.2 mm) in the femoral tunnel and one bioabsorbable interference screw (Mitek 8–10 mm) in the tibial tunnel (Fig. 2). After autograft positioning, PLG was applied into the femoral and tibial tunnels and into the graft itself (Fig. 3).

Rehabilitation

All patients followed the same rehabilitation protocol, with permission for immediate weight bearing and full range of motion [16]. No rehabilitation brace was used postoperatively. The importance of reaching full extension was emphasized from the beginning. Closed kinetic chain exercises were started immediately after the surgical procedure. Running was allowed at 12 weeks and contact sports at 6 months in cases with no knee-joint effusion, full range of motion and obtained muscle strength of 90% compared with the contralateral leg.

Follow-up evaluation

All patients were evaluated clinically and for knee stability before surgery, and at 3 and 6 months after surgery. Clinical evaluations were assessed using the Tagner activity score, Lyshol score and IKDC score [19, 20]. Objective anteroposterior knee stability was measured using the KT-2000 arthrometer at 15, 20 and 30 pounds of force (67 N, 89 N, 136 N) with knee flexion at 25° and fixed patella at the same time [21].

Statistical methods

Background data statistics included percentage distributions, mean values and standard deviations. Differences in patient characteristics in the two treatment groups were compared using Chi-squared tests for categorical variables and Mann–Whitney rank tests for continuous variables. The differences in arthrometer measurements before surgery and at the 3- and 6-month follow-ups was calculated to determine differences between the basic and the advanced treatment. The linear regression method was used to adjust the impact of basic and advanced treatment by age, BMI and arthrometer measurement before operation.

Results

Overall, 23 patients from the initial 25 (92%) in the control group and 22 of the initial 25 (88%) in the PLG group were available for the 3- and 6-month follow-ups; five patients were lost to follow-up for logistical reasons. Comparison between the PLG group and the control group in relation to preoperative parameters showed that the groups were similar (Table 2). In the PLG group the average platelet concentration in platelet-leukocyte-rich plasma was 962 (552–1326) G/l; the average blood platelet concentration in all patients was 192 G/l.

Table 2. Background data of patients with knee injuries

Characteristics	Control group ($n=23$)	Platelet group ($n=22$)	*P*-value
Sex (%)			0.353
male	73.9	59.1	
female	26.1	40.9	
Age in years	33.0±12.5	35.4±10.0	0.493
Injured knee (%)			1.000
right	56.5	59.1	
left	43.5	40.9	
BMI	24.5±2.3	26.2±4.2	0.098

Table 3. Evaluation of anteroposterior knee stability with the KT-2000 arthrometer

Characteristics	Control group ($n=23$)	Platelet gel group ($n=22$)	*P*-value
KT-2000 value (mm) before operation, load 136 N	7.9±2.7	7.9±2.9	0.925
KT-2000 value (mm) at 3-month follow-up, load 136 N	6.1±2.1	4.9±1.8	0.035
KT-2000 value (mm) at 6-month follow-up, load 136 N	6.7±2.1	4.7±1.9	0.003

Table 4. Linear model for calculation of the KT-2000 value at the 6-month follow-up

	Beta	Significance	95% CI
Therapy method	–0.452	<0.001	–2.979, –0.970
KT-2000 value before operation	0.557	<0.001	0.270, 0.632
Age	0.023	0.844	–0.041, 0.050
BMI	0.074	0.538	–0.106, 0.200

($R=0.719$, $R^2=0.518$).

The aim of our study was to determine the difference between the PLG and control groups regarding anteroposterior stability of the knee joint measured using the KT-2000 arthrometer (Table 3). The calculated 3-month (136 N) improvement was 1.8±1.7 mm in the control group and 3.0±2.5 mm in the PLG group, the difference being nonsignificant ($P=0.119$).

The calculated 6-month (136 N) improvement was 1.3±1.8 mm in the control group and 3.1±2.5 mm in the PLG group, resulting in a significant difference between the treatments ($P=0.011$).

We used the linear model for calculation of age, BMI, KT-2000 measurement before surgery, and the KT-2000 value at the 6-month point (independent variable) in relation to the method of treatment (control group vs. PLG group). Table 4 shows that the significant variables were the method of treatment and the KT-2000 value before operation, whereas age and BMI were not in any correlation with the KT-2000 value at the 6-month point.

Discussion

Arthroscopic findings and clinical results of ACL reconstructions with BTB or HT grafts are usually found to be satisfactory in both aggressive and low-aggressive rehabilitation programs [22]. There are no significant differences between BTB and HT groups concerning function scores (Tagner, Lysholm, IKDC); however, knee joints reconstructed with HT grafts are more lax than those reconstructed with BTB grafts. An additional issue is the long period of rehabilitation (6–9 months), due to processes of graft healing into the bone tunnels and ligamentization. Further, clinical studies have indicated that early return to vigorous physical activity may cause or increase the risk of greater knee laxity after ACL reconstruction [23].

The main reason for the lengthy rehabilitation after ACL reconstruction is the very slow graft healing in bone tunnels and very slow process of ligamentization of the intra-articular part of the graft. Ligamentization is the biomechanical and histological remodelling of the graft tissue from tendonous to ligamentous appearing in the new intra-articular environment specific to the native ACL [24]. Tendons used for ACL reconstruction appear similar to native ACL at arthroscopy and by observation under an optical microscope, but the electron microscope demonstrates that collagen fibrils in the grafts are organized differently from those of native ACL [25]. Since the center of the transplanted tissue is initially avascular and contains a relatively low number of viable cells, collagen synthesis cannot be very active in the early postoperative months, even though vascular invasion from the surface of the graft occurs within 3–8 weeks after the reconstruction and is followed by the repopulation phase [26]. Sufficient vascularization, mechanical forces, and release of growth factors that enter graft tissue via newly formed vessels all stimulate collagen production and maturation.

In our study we have demonstrated that PDGFs applied locally can improve knee stability in the first 3-month period and especially in the second 3-month period. We believe that the delivery of PDGFs mimics and accelerates physiological healing and reparative tissue processes in both graft healing and the graft ligamentization process. We have demonstrated that treatment with locally applied PLG could improve knee stability and also shorten the period of rehabilitation after reconstructive knee surgery.

We aim to explore further the role of locally applied PDGFs, particularly in relation to the early phase of graft revascularization.

Source of support
No external funding was received for this study. The study was approved by the National Ethics Committee.

Conflict of interest
The authors declare no conflict of interest.

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